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AGRICULTURAL IRRIGATION DRAINWATER STUDIES
in support of the
SAN JOAQUIN VALLEY DRAINAGE PROGRAM

ANNUAL REPORT
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INTRODUCTION

In March 1986 the U.S. Fish and Wildlife Service (USFWS) and the U.S. Bureau of Reclamation entered into an intra-agency agreement (6-AA-20-04170) for the purpose of supporting research on the effects of agricultural drainwater contaminants on fish and wildlife populations. The agreement initiated a multi-year research effort to be conducted by USFWS research centers in support of the San Joaquin Valley Drainage Program.

The National Fisheries Contaminant Research Center (NFCRC) received responsibility for seventeen major studies included in this agreement. Central to this research thrust is the determination of contaminant movement and localization in aquatic ecosystems, particularly the phenomenon of bioaccumulation and resultant exposure of fish and wildlife through the food chain. Study design includes both field and laboratory methodologies in order to isolate and identify specific chemical, physical, and biological factors influencing contaminant behavior and toxicity. All of the studies are interrelated to provide a comprehensive approach to elucidating the impacts of drainwater contaminants on fish and wildlife populations. This report summarizes research findings of NFCRC during fiscal year 1988.

FISH COMMUNITY COMPOSITION IN THE IRRIGATED SAN JOAQUIN VALLEY

Introduction

Crop production is threatened in about 162,000 ha of irrigated farmland on the San Joaquin Valley floor of California, where groundwater tables are shallow and saline. However, within 100 yr, the area of land requiring drainage could reach 405,000 ha, or slightly more than 22% of the total irrigated land on the Valley floor. Subsurface (tile) drainage systems have been installed on some Valley farms to remove excess groundwater and allow freshwater to leach salts from the soils. Currently, this drainage water is either disposed in small "on-farm" evaporation ponds or discharged into the San Joaquin River and its tributaries.

This study was part of a broader investigation designed to document the contaminant loads in resident and anadromous fishes that inhabit the San Joaquin River system. Specific objectives of the study were (i) to document the composition and abundance of fish faunas from representative sites; (ii) to document the physiochemical conditions occurring at each of the sites; and (iii) to determine if any changes in fish faunas among the various sites were related to variations in physiochemical conditions.

Methods

Fish were sampled from 26 sites in the San Joaquin Valley between September and December 1986. At each site, fish were captured with a variety of gear (beach seines, bag seines, gill nets, and backpack electrofishing gear), identified to species, counted, then returned to the water. To compute catch-per-effort statistics, we standardized either the amount of area fished (i.e., beach and bag seining) or the time spent fishing (i.e., gill netting and electroshocking).

At each site, we measured the following environmental variables: current velocity, water temperature, pH, turbidity, dissolved oxygen, total alkalinity, total hardness, conductivity, total dissolved solids, stream width, stream depth, and the particle size distribution of bottom sediments. We also estimated the percentage of pools, riffles, and runs, and the percentage of cover provided by emergent and submerged vegetation. Finally, we assigned each site a subjective rating of 1-5 (1 being the lowest) that characterized the extent of "human impact" from channelization, removal of riparian cover, and water flow diversions.

Progress

A total of 34,901 fish representing 35 species, 26 genera, and 15 families were captured during the study (Table 1). Introduced fishes made up most of the samples with less desirable species--such as threadfin shad, inland silversides, mosquitofish, and red shiners--contributing >75% of the total catch (Table 1). These less desirable species were most abundant in irrigation canals and drains, and other shallow, emphemeral, and unstable aquatic habitats strongly influenced by agricultural drainage activities. Bluegill and other centrarchids, which are generally regarded as sensitive to selenium toxicity, were rare or absent in many tributaries of the San Joaquin River that received inflows made up almost entirely of agricultural tile drainwater. Significant correlations between fish density and the environmental variables indicated that introduced species were mostly associated with water temperature, pH, dissolved oxygen, conductivity, turbidity, alkalinity, hardness, and total dissolved solids (Table 2). These environmental variables are most influenced by agricultural drainwater. The associations between the abundance of introduced species and these environmental variables may become more prominent as aquatic habitats on the San Joaquin Valley floor receive greater contributions of drainwater from various agricultural sources.

Work Remaining

Statistical analysis of the relationships between fish community composition and water quality variables will continue in order to further evaluate the effects of agricultural drainwater on the fish fauna of the San Joaquin Valley. We are also attempting to document changes in the Sacramento-San Joaquin Valley ichthyofauna in relation to changing land-use patterns--especially agriculture--both recently (since 1970) and over the past 100 years.

Table 1. Percentage and number of fishes collected from 26 sites in the San Joaquin Valley, California. Asterisks identify introduced species.

Species code (scientific name)	Percentage of sites where present	Total number collected	Percent occurrence of of individuals in all samples
AS (*Alosa sapidissima*)*	23	11	0.03
BB (*Ictalurus nebulosus*)*	15	30	0.09
BC (*Promoxis nigromaculatus*)*	77	250	0.72
BG (*Lepomis macrochirus*)*	96	1,332	3.82
BLB (*Ictalurus melas*)*	46	188	0.54
CCF (*Ictalurus punctatus*)*	65	151	0.43
CP (*Cyprinus carpio*)*	100	696	1.99
CS (*Oncorhynchus tshawytscha*)	4	1	<0.01
FHM (*Pimephales promelas*)*	65	1,213	3.48
GB (*Orthodon microlepidotus*)	69	625	1.79
GF (*Carassius auratus*)*	85	707	2.03
GMB (*Gambusia affinis*)*	92	2,497	7.15
GS (*Lepomis cyanellus*)*	100	387	1.11
GSH (*Notemigonus crysoleucas*)*	46	27	0.08
HCH (*Lavinia exilicauda*)	46	56	0.16
ISS (*Menidia beryllina*)*	69	3,060	8.77
LM (*Lampetra* spp.)	4	6	0.02
LMB (*Micropterus salmoides*)*	92	468	1.34
LP (*Percina macrolephus*)*	62	132	0.38
RE (*Lepomis microlophus*)*	65	477	1.37
RSH (*Notropis lutrensis*)*	62	1,341	3.84
SB (*Morone saxatilis*)*	69	291	0.83
SCP (*Cottis asper*)	23	109	0.31
SMB (*Micropterus dolomieui*)*	19	46	0.13
SQ (*Ptychocheilus grandis*)	8	9	0.03
SS (*Catostomus occidentalis*)	26	19	0.05
ST (*Pogonichthys macrolepidotus*)	4	13	0.04
TFS (*Dorosoma petenense*)*	88	20,228	57.96
TP (*Hysterocarpus traskii*)	12	281	0.81
WC (*Promoxis annularis*)*	62	118	0.34
WCF (*Ictalurus catus*)*	81	105	0.30
WM (*Lepomis gulosus*)*	27	19	0.05
WS (*Acipenser transmontanus*)	4	1	<0.01
YF (*Acanthogobius flavimanus*)*	8	5	0.01
CR (*Lavinia symmetricus*)	4	2	0.01

Table 2. Geometric means of environmental variables from sites in the San Joaquin Valley where introduced fish species were captured. Refer to Table 1 for fish species codes. Levels of significance (*$\underline{P}\leq 0.05$; **$\underline{P}\leq 0.01$) are determined by Spearman's rank correlations, r_s. Means that are underlined indicate negative correlations.

Environmental variable	Fish species AS	BC	BG	BLB	CCF
Water temperature (°C)	$\underline{17.8}$	18.8	18.4	$\underline{18.8}$	19.1
Current velocity (m/sec)	0.3	0.3	$\underline{0.2}$	0.2	$\underline{0.2}$*
pH	$\underline{7.4}$	$\underline{7.4}$	7.3	7.5	7.5
Dissolved oxygen (mg/l)	8.6	$\underline{8.4}$	$\underline{8.5}$	$\underline{8.5}$	8.4
Conductivity (µmhos/cm @ 25°C)	$\underline{468.7}$	641.0	$\underline{626.6}$	703.1	631.8
Turbidity (NTU's)	$\underline{7.4}$	8.7*	$\underline{7.4}$	6.8	$\underline{7.1}$
Total alkalinity (mg/l as $CaCO_3$)	$\underline{79.2}$	$\underline{88.4}$	$\underline{84.7}$	92.5	88.7
Total hardness (mg/l as $CaCO_3$)	$\underline{109.0}$	$\underline{153.9}$	$\underline{148.8}$	168.0	151.8
Total dissolved Solids (mg/l)	$\underline{264.5}$	$\underline{404.7}$	$\underline{395.6}$	459.3	$\underline{402.4}$
Average depth (m)	1.1	1.3**	1.2	$\underline{1.0}$	$\underline{0.8}$**
Greatest depth (m)	1.9	2.1	2.0	$\underline{1.8}$	$\underline{1.5}$
Width (m)	38.0	59.1**	146.5**	$\underline{270.9}$	$\underline{196.4}$
Emergent vegetation (%)	5.8	$\underline{7.7}$	7.6	10.5	9.4
Submergent vegetation (%)	$\underline{3.3}$	$\underline{4.6}$	$\underline{5.9}$	$\underline{8.4}$	7.9
Pool (%)	$\underline{16.7}$	$\underline{20.3}$	27.8	$\underline{37.9}$	32.6
Riffle (%)	10.8	$\underline{9.3}$	11.0	$\underline{9.6}$	11.2
Run (%)	72.5	70.5	$\underline{61.2}$	52.5	$\underline{56.2}$*
Human impact (1-5)	3.3	$\underline{3.2}$	$\underline{3.1}$	$\underline{3.2}$	3.2
Sediment factor (0-1)	0.2	$\underline{0.2}$	$\underline{0.2}$	$\underline{0.2}$	$\underline{0.2}$

Table 2.--Continued.

Environmental variable	Fish species				
	CP	FHM	GF	GMB	GS
Water temperature (°C)	18.5	19.4	18.5	18.3	18.5
Current velocity (m/sec)	0.2	0.2	0.3	0.3	0.2
pH	7.4	7.6**	7.4	7.3	7.4
Dissolved oxygen (mg/l)	8.5	8.3	8.4	8.5*	8.5
Conductivity (μmhos/cm @ 25°C)	625.7**	817.0**	689.3**	647.8**	625.7
Turbidity (NTU's)	7.7	10.2**	8.0	7.8*	7.7
Total alkalinity (mg/l as $CaCO_3$)	84.6**	103.2**	91.4**	85.4**	84.6
Total hardness (mg/l as $CaCO_3$)	148.8**	192.9**	162.6**	153.1**	148.8
Toatal dissolved Solids (mg/l)	394.5**	521.8**	435.1**	410.6**	394.5
Average depth (m)	1.1	1.1**	1.2	1.1**	1.1*
Greatest depth (m)	2.0	1.7**	2.2	1.9**	2.0*
Width (m)	141.0	56.5**	161.3	35.5	141.0
Emergent vegetation (%)	7.5	8.7	8.2**	7.8	7.5
Submergent vegetation (%)	5.6	4.9	5.4	5.4	5.7
Pool (%)	26.7*	18.5**	23.9	20.6*	26.7
Riffle (%)	10.6	6.8	9.5	11.5	10.6
Run (%)	62.7	74.7**	66.6	67.9	62.7
Human impact (1-5)	3.2	3.5*	3.1	3.1	3.2
Sediment factor (0-1)	0.2	0.1*	0.2	0.2	0.2

Table 2.--Continued.

Environmental variable	Fish species GSH	ISS	LMB	LP	RE
Water temperature (°C)	17.7	18.8	18.6	19.4**	19.0
Current velocity (m/sec)	0.3	0.2	0.3	0.2	0.3
pH	7.3	7.6	7.3	7.5	7.3*
Dissolved oxygen (mg/l)	8.5	8.4	8.4	8.2**	8.6
Conductivity (µmhos/cm @ 25°C)	731.2	658.7	590.3	708.3	348.7**
Turbidity (NTU's)	7.7	7.8	8.0	8.8	6.2
Total alkalinity (mg/l as $CaCO_3$)	91.1	92.2	83.7*	96.0	61.9**
Total hardness (mg/l as $CaCO_3$)	167.4	155.2	143.4*	166.2	89.6**
Total dissolved Solids (mg/l)	457.5	415.1	374.0*	442.2	210.2**
Average depth (m)	1.0	1.2	1.2	1.3	1.4
Greatest depth (m)	1.7	2.1	2.0	2.2	2.4
Width (m)	236.7	195.0	52.0	213.3	65.3*
Emergent vegetation (%)	5.9	8.2	8.0	7.5	5.5
Submergent vegetation (%)	6.4	4.9	4.6	7.0	4.1
Pool (%)	31.7	26.9	24.0	25.3	28.5
Riffle (%)	12.5	8.6	10.6	8.1	11.8
Run (%)	55.8	64.4	65.4	66.6	59.7
Human impact (1-5)	3.3	3.3	3.1	3.3	2.9
Sediment factor (0-1)	0.2	0.2	0.2	0.2	0.2

Table 2.--Continued.

Environmental variable	Fish species RSH	SB	TFS	WC	WCF
Water temperature (°C)	18.6	18.9	18.7	19.4**	18.3
Current velocity (m/sec)	0.3	0.2	0.2	0.2	0.2
pH	7.6*	7.5**	7.5**	7.5	7.5
Dissolved oxygen (mg/l)	8.3	8.3	8.4	8.2*	8.5
Conductivity (μmhos/cm @ 25°C)	880.2**	753.2*	698.1*	617.8	584.4
Turbidity (NTU's)	10.1**	8.6	8.6	8.4	6.4
Total alkalinity (mg/l as $CaCO_3$)	106.1**	96.4*	94.5	91.9	82.2
Total hardness (mg/l as $CaCO_3$)	200.1**	172.7*	164.6*	149.6	139.8
Total dissolved Solids (mg/l)	549.6**	467.1	440.2	390.4	370.0
Average depth (m)	1.1	1.3	1.2	1.3	1.1
Greatest depth (m)	1.5*	2.2	2.0	2.0	2.0
Width (m)	33.0*	193.1	156.5	66.0	170.2
Emergent vegetation (%)	6.2	5.6	7.9	7.4	8.1
Submergent vegetation (%)	4.6	4.9	5.1	4.1	6.3
Pool (%)	16.6	26.1	23.7	24.7	31.4
Riffle (%)	9.1	8.1	8.3	9.4	11.7*
Run (%)	74.4**	65.8	68.0	65.9	56.9
Human impact (1-5)	3.4	3.3*	3.3	3.3	3.0
Sediment factor (0-1)	0.2	0.2	0.2	0.2	0.2

SYNOPTIC SURVEY OF CONTAMINANTS IN SAN JOAQUIN RIVER FISHES

Introduction

Recent contaminant surveys in the San Joaquin Valley indicate that some fish populations contain high levels of selenium and other inorganic and organic chemicals, but the geographic (spatial) patterns and severity of contamination are still poorly defined. The contaminant concentrations in San Joaquin River and San Francisco Estuary fish populations must be determined not only to identify "hot spots" and establish current baseline conditions for subsequent surveys, but also to provide reference points for field and laboratory studies that examine cause-and-effect relationships between contaminant burdens and biological impacts.

Specific objectives of this study are (i) to identify the locations where selenium and other trace element concentrations in fish have reached levels of environmental concern; (ii) to determine if trace element concentrations in fish vary according to species, sex, and tissue type (fillets, gonads, carcass); and (iii) to determine if trace element concentrations have increased in San Joaquin River fish populations since 1981 due to recent changes in the management and use of agricultural subsurface (tile) drainwater in the Valley.

Methods

Samples of up to 7 fish species were collected either in September-December 1986 (carp, mosquitofish, bluegills, largemouth bass, striped bass, and Sacramento blackfish), or in February-April 1987 (chinook salmon fingerlings), or in July-August 1987 (striped bass fingerlings) from up to 27 sites in the San Joaquin Valley, 1 site in the Sacramento Valley, and up to 3 sites in the San Francisco Estuary. The fish were collected with trawls, seines, gill nets, and electrofishing gear. In addition, bluegills and largemouth bass from selected sites were segregated by sex and subsampled for fillets, gonads (bluegills only), and the remaining carcasses. The samples were wrapped and bagged in plastic, frozen, then shipped to analytical laboratories for analysis of trace elements.

Progress

A total of 379 samples were collected during this investigation, of which 324 were shipped to NFCR-Columbia where they are still undergoing sample preparation and analysis for boron, molybdenum, and selenium. The remaining 55 samples--consisting entirely of subadult (≤445 mm total length) striped bass collected from 19 sites in the Valley and 3 sites in the Estuary (see Figure 1) in September-December 1986--were shipped to the Patuxent Analytical Facility in Laurel, MD, for whole-body analysis of arsenic, mercury, and selenium, plus a scan of 16 other elements (including boron and molybdenum). Data from the subadult striped bass are summarized below.

With one exception, all trace elements were detected in 1 or more of the 55 samples of striped bass collected during this survey (Table 1). The geometric mean concentrations (µg/g dry weight; ranges in parentheses) were as follows: aluminum, 24.1 (not detected, ND-460.); arsenic, 0.367 (ND-1.6); boron, 9.82 (ND-18.); barium, 4.37 (0.51-98.); cadmium, 0.196 (ND-0.36); chromium, 1.78 (ND-13.); copper, 1.30 (ND-12.); iron, 49.6 (17.-500.); lead, 0.396 (ND-1.3); and magnesium, 619. (16.-2100.). Also, mercury, 0.153 (ND-0.48); molybdenum, 0.202 (ND-1.7); nickel, 0.682 (ND-4.2); selenium, 2.64 (ND-7.9); strontium, 50.4 (18.-200.); tin, 4.65 (ND-66.); vanadium, 0.196 (ND-0.33); and zinc, 46.5 (25.-170.). The exception was beryllium, which was never detected in the samples.

When compared to the 85th percentile concentrations in whole freshwater fish measured by the National Contaminant Biomonitoring Program (NCBP) or other contaminant surveys, a few samples of striped bass contained moderately elevated levels of aluminum, arsenic, cadmium, copper, and selenium. In addition, the mean concentrations of 8 elements--aluminum, arsenic, copper, iron, magnesium, selenium, strontium, and tin--differed significantly ($\underline{P} \leq 0.05$) among sites (Table 1). However, only selenium exhibited high concentrations (up to 7.9 µg/g) in samples from Valley sites exposed to tile drainwater, with lower concentrations in samples collected elsewhere. Water quality variables--especially those that are strongly influenced by tile drainwater (e.g., conductivity, total dissolved solids, total alkalinity, and total hardness)--also were significantly correlated ($\underline{P} \leq 0.05$) only with the selenium concentrations in fish (Table 2).

Although selenium levels in striped bass from drainwater-impacted reaches of the San Joaquin River and its tributaries approached or exceeded the highest concentrations reported by the NCBP, it is unknown if the concentrations adversely affected their survival. Selenium levels in striped bass from the Estuary (generally <2 $\mu g/g$; see Table 1) were 1/4-1/2 the mean concentrations measured in fish from the most contaminated reaches of the San Joaquin River.

Work Remaining

A manuscript describing the findings from our survey of trace elements in subadult striped bass is currently being finalized for internal (NFCR-Columbia) review. In addition, we are waiting for the analytical results from our collections of resident freshwater fishes and the fingerling life stages of chinook salmon and striped bass. After receiving these results, comparisons with residue levels reported in published and unpublished "cause-and-effect" studies will be used to determine if the trace element concentrations in fish tissues have reached toxic levels. If potentially toxic levels are found, follow-up studies will be proposed to document any adverse impacts on fish populations.

Table 1. Trace element concentrations ($\mu g/g$ dry weight) in striped bass from the San Joaquin Valley and San Francisco Estuary, September-December, 1986. N, sample size (no. detected/no. analyzed); M, mean (value is geometric mean if ≥2 samples analyzed); r, range; ND, not detected.[a]

Region, site[b], and Kruskal-Wallis test statistic[c]	Al			As			Ba		
	N	M	R	N	M	R	N	M	R
Upper San Joaquin River:									
1	3/3	7.56	5.6-9.8	1/3	0.275	ND-0.41	3/3	6.11	5.7-6.5
2	1/1	17.	—	1/1	0.56	—	1/1	4.0	—
3	2/2	28.9	8.2-100.	0/2	ND	ND-ND	2/2	5.54	4.5-6.8
4	2/3	7.33	ND-24.	1/3	0.26	ND-0.45	3/3	2.43	1.3-4.6
Middle San Joaquin River:									
5	2/2	152.	89.-260.	1/2	0.297	ND-0.45	2/2	3.28	3.2-3.3
6	2/3	10.7	ND-28.	1/3	0.242	ND-0.35	3/3	3.61	2.9-4.5
Lower San Joaquin River:									
7	1/2	2.92	ND-4.4	0/2	ND	ND-ND	2/2	2.51	1.7-3.8
8	1/1	53.	—	1/1	0.41	—	1/1	7.0	—
9	1/1	460.	—	1/1	0.54	—	1/1	17.	—
10	2/3	7.60	ND-25.	3/3	0.652	0.56-0.87	3/3	3.61	1.8-6.8
Southern tributaries:									
11	3/3	35.9	27.-64.	1/3	0.260	ND-0.41	3/3	3.44	3.0-3.9
12	3/3	36.7	18.-78.	0/3	ND	ND-ND	3/3	8.29	6.2-9.7
Grassland tributaries:									
13	1/1	46.	—	0/1	ND	—	1/1	11.	—
14	1/1	240.	—	0/1	ND	—	1/1	13.	—
15	10/10	58.9	6.1-150.	3/10	0.242	ND-0.59	10/10	5.02	2.0-98.
16	1/1	62.	—	0/1	ND	—	1/1	3.1	—

Table 1.—Continued.

Region, site[b], and Kruskal-Wallis test statistic[c]	Cr			Cu			Fe		
	N	M	R	N	M	R	N	M	R
Grassland tributaries:									
17	2/2	10.9	4.1-29.	1/2	0.309	ND-0.56	2/2	0.937	0.51-1.7
18	3/3	63.2	55.-79.	2/3	0.377	ND-0.58	3/3	1.56	1.4-1.7
Eastern tributary:									
19	1/1	14.	—	1/1	0.41	—	1/1	4.1	—
San Francisco Estuary:									
20	3/3	7.64	6.0-11.	3/3	1.32	1.2-1.6	3/3	6.07	3.4-8.8
21	2/3	9.22	ND-26.	3/3	1.23	1.2-1.3	3/3	5.97	4.1-9.8
22	3/3	14.4	10.-18.	3/3	1.44	1.3-1.5	3/3	3.92	2.0-7.9
X^2 (df=21)		38.128*			39.707**			32.055	
Upper San Joaquin River:									
1	1/3	2.18	ND-9.3	3/3	1.77	1.3-2.2	3/3	29.2	18.-51.
2	1/1	2.1	—	1/1	1.8	—	1/1	36.	—
3	2/2	7.05	3.8-13.	2/2	2.62	2.4-2.9	2/2	64.7	31.-140.
4	1/3	1.24	ND-2.4	3/3	2.23	1.9-2.7	3/3	25.1	20.-37.
Middle San Joaquin River:									
5	1/2	1.5	ND-2.5.	2/2	2.11	1.6-2.7	2/2	149.	110.210.
6	2/3	2.09	ND-3.6	3/3	2.19	1.6-3.6	3/3	34.2	28.-39.
Lower San Joaquin River:									
7	0/2	ND	ND-ND	2/2	1.52	1.0-2.2	2/2	25.1	24.-26.
8	1/1	5.8	—	0/1	ND	—	1/1	90.	—
9	1/1	6.6	—	1/1	4.6	—	1/1	500.	—
10	0/3	ND	ND-ND	1/3	0.668	ND-2.5	3/3	25.0	18.-41.

Table 1.—Continued.

Region, site[b], and Kruskal-Wallis test statistic[c]	Cr			Cu			Fe		
	N	M	R	N	M	R	N	M	R
Southern tributaries:									
11	3/3	5.63	3.5-7.2	3/3	1.71	1.7-1.8	3/3	58.4	45.-85.
12	1/3	1.43	ND-2.9	0/3	ND	ND-ND	3/3	56.9	34.-91.
Grassland tributaries:									
13	0/1	ND	—	1/1	12.	—	1/1	300.	—
14	1/1	4.6	—	1/1	3.4	—	1/1	200.	—
15	5/10	1.53	ND-4.0.	9/10	1.34	ND-2.1	10/10	65.1	24.-120.
16	1/1	7.1	—	1/1	2.3	—	1/1	84.	—
17	1/2	1.42	ND-2.4	0/2	ND	ND-ND	2/2	27.4	17.-44.
18	1/3	1.23	ND-2.4	3/3	2.02	1.6-2.5	3/3	66.9	59.-79.
Eastern tributary:									
19	0/1	ND	—	1/1	1.8	—	1/1	22.	—
San Francisco Estuary:									
20	0/3	ND	ND-ND	2/3	0.901	ND-1.5	3/3	29.9	28.-34.
21	2/3	1.63	ND-2.6	2/3	0.919	ND-1.5	3/3	37.9	24.-55.
22	1/3	1.30	ND-2.5	0/3	ND	ND-ND	3/3	34.8	27.-45.
X^2 (df=21)		29.334			37.112*			35.618*	
Upper San Joaquin River:									
1	1/3	0.133	ND-0.20	3/3	835.	610.-1200.	2/3	0.646	ND-2.5
2	1/1	0.32	—	1/1	860.	—	1/1	0.64	—
3	1/2	0.168	ND-0.27	2/2	856.	820.-890.	2/2	2.21	1.2-4.2
4	3/3	0.218	0.16-0.32	3/3	689.	610.-760.	2/3	0.487	ND-1.3
Middle San Joaquin River:									
5	1/2	0.158	ND-0.26	2/2	674.	540.-830.	2/2	1.04	1.0-1.1

Table 1.—Continued.

Region, site[b], and Kruskal-Wallis test statistic[c]	Cr			Cu			Fe		
	N	M	R	N	M	R	N	M	R
Middle San Joaquin River:									
6	2/3	0.205	ND-0.33	3/3	811.	760.-890.	3/3	1.05	0.89-1.4
Lower San Joaquin River:									
7	2/2	0.330	0.23-0.48	2/2	505.	500.-510.	0/2	ND	ND-ND
8	1/1	0.26	—	1/1	530.	—	1/1	2.2	—
9	0/1	ND	—	1/1	1200.	—	1/1	3.6	—
10	3/3	0.199	0.18-0.22	3/3	471.	430.-530.	1/3	0.237	ND-0.45
Southern tributaries:									
11	0/3	ND	ND-ND	3/3	845.	810.-870.	3/3	1.76	1.1-2.8
12	0/3	ND	ND-ND	3/3	590.	550.-650.	2/3	0.564	ND-1.3
Grassland tributaries:									
13	0/1	ND	—	1/1	2100.	—	0/1	ND	—
14	0/1	ND	—	1/1	16.	—	1/1	1.7	—
15	2/10	0.111	ND-0.34	10/10	633.	490.-750.	8/10	0.641	ND-1.7
16	1/1	0.33	—	1/1	840.	—	1/1	2.2	—
17	1/2	0.122	ND-0.15	2/2	480.	440.-520.	1/2	0.396	ND-0.93
18	3/3	0.246	0.19-0.31	3/3	695.	680.-720.	3/3	0.683	0.64-0.72
Eastern tributary:									
19	0/1	ND	—	1/1	660.	—	1/1	0.35	—
San Francisco Estuary:									
20	0/3	ND	ND-ND	3/3	520.	500.-560.	2/3	0.398	ND-0.76
21	2/3	0.202	ND-0.31	3/3	546.	520.-590.	3/3	0.805	0.49-1.1
22	2/3	0.156	ND-0.24	3/3	551.	530.-560.	3/3	0.600	0.35-0.87
X^2 (df=21)			29.946			43.352**			30.526

Table 1.—Continued.

Region, site[b], and Kruskal-Wallace test statistic[c]	Se			Sn			Sr			Zn		
	N	M	R	N	M	R	N	M	R	N	M	R
Upper San Joaquin River:												
1	3/3	1.85	1.5-2.2	2/3	4.02	ND-6.0	3/3	47.3	39.-60.	3/3	42.0	25.79.
2	1/1	3.4	—	1/1	6.4	—	1/1	33.	—	1/1	51.	—
3	2/2	2.67	2.6-2.8	2/2	9.99	5.3-19.	2/2	44.8	39.-51.	2/2	50.1	39.64.
4	3/3	1.66	0.61-3.5	2/3	3.60	ND-6.6	3/3	30.7	18.-42.	3/3	37.9	31.54.
Middle San Joaquin River:												
5	2/2	5.76	5.7-5.8	2/2	13.4	5.4-33.	2/2	44.1	42.-47.	2/2	48.0	45.51.
6	3/3	3.53	2.7-5.4	3/3	5.65	4.6-6.4	3/3	52.1	42.-66.	3/3	42.5	31.51.
Lower San Joaquin River:												
7	2/2	2.11	2.0-2.2	0/2	ND	ND-ND	2/2	37.5	36.-39.	2/2	35.8	28.47.
8	1/1	2.7	—	1/1	4.9	—	1/1	58.	—	1/1	58.	—
9	1/1	2.9	—	1/1	66.	—	1/1	87.	—	1/1	83.	—
10	3/3	1.03	0.66-2.0	0/3	ND	ND-ND	3/3	49.4	36.-64.	3/3	39.1	27.49.
Southern tributaries:												
11	2/3	0.641	ND-1.2	3/3	9.28	7.3-13.	3/3	46.5	45.-48.	3/3	63.3	57.70.
12	3/3	2.58	2.2-2.8	0/3	ND	ND-ND	3/3	53.7	50.-56.	3/3	56.3	50.65.
Grassland tributaries:												
13	1/1	3.4	—	0/1	ND	—	1/1	200.	—	1/1	170.	—
14	1/1	4.6	—	1/1	29.	—	1/1	60.	—	1/1	74.	—
15	10/10	4.89	4.2-6.3	7/10	6.80	ND-19.	10/10	39.8	33.-53.	10/10	36.9	28.53.
16	1/1	2.7	—	1/1	12.	—	1/1	37.	—	1/1	58.	—
17	2/2	5.86	5.6-6.1	0/2	ND	ND-ND	2/2	44.1	37.-52.	3/3	40.6	31.52.
18	3/3	6.88	5.7-7.9	3/3	9.86	8.6-12.	3/3	52.1	48.-55.	3/3	37.1	34.39.

Table 1.—Continued.

Region, site[b], and Kruskal-Wallace test statistic[c]	Se			Sn			Sr			Zn		
	N	M	R	N	M	R	N	M	R	N	M	R
Eastern tributary:												
19	1/1	3.2	—	1/1	4.1	—	1/1	48.	—	1/1	37.	—
San Francisco Estuary:												
20	3/3	1.27	0.86-1.9	0/3	ND	ND-ND	3/3	81.2	79.-84.	3/3	51.8	46.56.
21	3/3	1.73	1.4-2.0	1/3	2.38	ND-3.9	3/3	76.0	71.-79.	3/3	51.7	45.63.
22	3/3	1.92	1.2-3.3	0/3	ND	ND-ND	3/3	73.9	60.-94.	3/3	55.0	52.60.
X^2 (df=21)		47.128**			38.433*			40.454**			30.646	

[a] Six elements (not shown) either were never detected (e.g., Be) or were detected in only 1 sample as follows (element, site, N, M, R): B, 22, 1/3, 11.7, ND-18.; Cd, 7, 1/2, 0.265, ND-0.36; Mo, 17, 1/2, 0.528, ND-1.7; Pb, 1, 1/3, 0.637, ND-1.3; and V, 15, 1/10, 0.188, ND-0.33.

[b] For each region, sampling sites are listed in approximately longitudinal (upstream-downstream) order. See Fig. 1 for names and locations of sites.

[c] Codes: * $\underline{P} \leq 0.05$; ** $\underline{P} \leq 0.01$.

Table 2. Spearman's rank correlation coefficients ($\underline{r}_s$) that describe the relations between the concentrations of 17 trace elements—aluminum (Al), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), magnesium (Mg), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), tin (Sn), strontium (Sr), vanadium (V), and zinc (Zn)—detected in striped bass, and selected physical and chemical parameters at 19 sites in the San Joaquin Valley. Codes: * $\underline{P} \leq 0.05$; ** $\underline{P} \leq 0.01$.

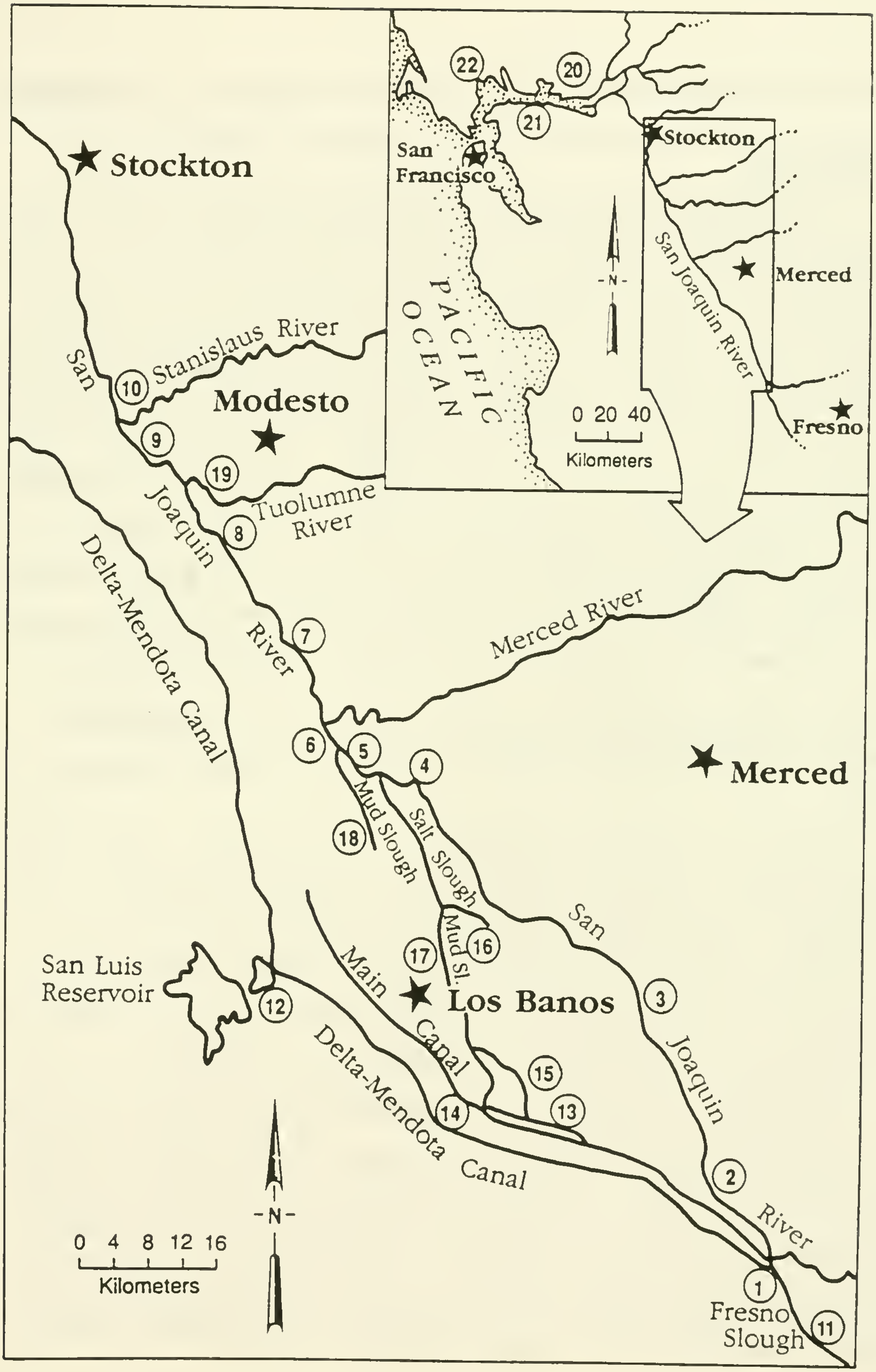
	Physical				
Trace element[a]	Depth	Current velocity	Turbidity	Conductivity	Temperature
Al	-0.55*	0.10	0.26	0.34	-0.01
As	0.01	0.54*	-0.42	-0.21	-0.36
Ba	0.15	0.10	0.17	-0.31	0.05
Cd	0.34	-0.04	-0.09	0.17	<0.01
Cr	-0.23	0.21	0.37	-0.12	0.25
Cu	-0.01	0.07	0.37	-0.13	0.36
Fe	-0.49*	0.10	0.41	0.38	0.18
Hg	-0.09	0.27	0.05	0.29	0.07
Mg	-0.08	-0.01	0.21	-0.25	0.50*
Mo	-0.34	-0.39	-0.26	0.34	-0.39
Ni	-0.27	0.32	0.25	0.18	0.13
Pb	0.26	-0.09	0.04	-0.30	0.34
Se	-0.74**	0.12	0.12	0.58**	-0.28
Sn	-0.37	0.43	0.33	0.18	0.18
Sr	0.07	-0.06	-0.15	-0.06	-0.31
V	-0.30	0.09	0.39	0.39	-0.04
Zn	-0.08	-0.03	0.34	-0.07	0.24

Table 2.—Continued.

Trace element[a]	Dissolved oxygen	pH	Total alkalinity	Total hardness	Total dissolved solids
	Chemical				
Al	0.09	0.21	0.22	0.25	0.27
As	-0.03	-0.48*	-0.33	-0.26	-0.21
Ba	0.12	0.01	-0.32	-0.32	-0.23
Cd	0.09	0.04	0.17	0.17	0.17
Cr	-0.31	0.07	0.16	0.10	0.12
Cu	0.08	-0.04	-0.20	-0.19	-0.15
Fe	-0.05	0.27	0.29	0.30	0.32
Hg	-0.40	-0.29	0.33	0.31	0.31
Mg	-0.23	-0.03	-0.20	-0.30	-0.29
Mo	-0.09	0.34	0.34	0.34	0.34
Ni	-0.15	0.02	0.16	0.13	0.16
Pb	-0.22	0.13	-0.13	-0.13	-0.09
Se	-0.11	0.21	0.52*	0.54*	0.62**
Sn	-0.06	-0.05	0.09	0.12	0.14
Sr	0.53*	0.19	-0.17	-0.13	-0.04
V	-0.17	0.09	0.39	0.39	0.39
Zn	0.02	0.19	-0.12	-0.13	-0.16

[a] Boron was omitted from this analysis because detectable concentrations were found in only 1 of 55 samples.

Figure 1. Names and locations of sampling sites in the study area. Sites on the San Joaquin River are as follows: (1) at Mendota Pool; (2) at Firebaugh; (3) at Highway 152; (4) at Lander Avenue; (5) at Fremont Ford State Recreation Area; (6) above Hills Ferry Road; (7) at Crows Landing Road; (8) at Laird Park; (9) at Maze Road; and (10) at Durham Ferry State Recreation Area. The remaining sites are: (11) Fresno Slough at the Mendota Wildlife Area; (12) Delta-Mendota Canal at O'Neil Forebay; (13) Helm Canal; (14) Main Canal; (15) Agatha Canal; (16) Salt Slough at Hereford Road; (17) Mud Slough at the Los Banos Wildlife Area; (18) Mud Slough at Gun Club Road; (19) Tuolumne River at Shiloh Road; (20) Honker Bay; (21) Suisun Bay; and (22) San Pablo Bay.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20
21
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Tuolumne River
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Merced River
Delta-Mendota Canal
6
5
4
Merced
Mud Slough
Salt Slough
18
Mud Sl.
16
17
San Luis Reservoir
12
Los Banos
Main Canal
3
San Joaquin River
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Delta-Mendota Canal
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ACUTE AND CHRONIC EXPOSURES OF SALMON TO SELENIUM IN FRESHWATER

Introduction

Contamination of Kesterson Reservoir and adjacent wetlands by selenium-laden irrigation drainage water has caused concern for potential adverse effects on fish and wildlife resources. Elevated water concentrations of selenium in subsurface drainage water already enters freshwater habitats of the Grasslands area located south and adjacent to Kesterson. Selenium-laden water enters the Grasslands by way of the Helm and Main canals and from smaller ditches and drains; water leaves the Grasslands through Mud Slough, which serves as the primary drainage system, and in turn, drains into the San Joaquin River. A second route of selenium entry into the San Joaquin River is by subsurface seepage from Kesterson Reservoir which is located adjacent to the river.

Entry of elevated concentrations of selenium into the San Joaquin River could potentially cause adverse effects on salmonids. Toxicological impacts on salmonids would occur primarily during the early life stages when fry migrate down the San Joaquin River from spawning areas in tributaries.

The present research was undertaken to determine the effects on chinook salmon of waterborne exposure to selenium in water qualities which simulate potential environmental conditions in freshwater. Toxicity studies are to be conducted with various forms of selenium, individually and in various mixtures that simulate reported environmental ratios.

Methods

The swimup life stage (0.5 g) of fall-run chinook salmon was used in one chronic toxicity study to determine the lethal and sublethal effects of a 120-day exposure to waterborne selenium. The forms of selenium tested were sodium selenate and sodium selenite, and the environmental ratio of selenate:selenite tested was 6:1. The chronic toxicity test was conducted in standardized test waters that simulated the major cation and anion concentrations (minus trace elements) in standardized San Luis Drain water diluted 10-fold in a standardized freshwater receiving water. Actual drainwater was not used in toxicity studies because its water quality varies with location and time of year.

The water quality of the exposure water was pH 7.8, 211 ppm hardness, 109 ppm alkalinity as HCO_3, 45 ppm calcium, 24 ppm magnesium, 70 ppm sodium, 60 ppm chloride, and 188 ppm sulfate. This reconstituted water quality was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with various salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate. The chronic toxicity test was conducted in an intermittent-flow proportional diluter which delivered 1 liter of reconstituted water to each exposure aquaria at 15 minute intervals. Six duplicate nominal concentrations of selenium were tested (0, 8, 17, 35, 70, and 140 ppb). Throughout the study, mortality was recorded daily, fish behavior checklist evaluated weekly, water samples collected weekly for measurement of eight water quality parameters, water samples collected every 15 days for measurement of selenium concentrations, and fish samples collected for measurement of selenium concentrations, fish length and weight measurements, video taping of fish behavior, and measurement of fish swimming performance were accomplished at 30-day intervals. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 15 fish from each treatment following the 120-day exposure.

Progress

Acute toxicity tests with early life stages of chinook salmon indicate that swimup fry were more sensitive to selenate and selenite than were the eyed-egg or alevin life stages (Table 1). Acute toxicity tests with two stocks of young chinook salmon from California tested in a reconstituted freshwater revealed no difference in their sensitivity to selenite and selenate (Table 2). The hazard potential of selenate and selenite to indigenous salmon populations was estimated by comparing the acute toxicity values (96-hour LC50 value: concentration that kills 50% of the test animals in a 96-hour time period) with the expected environmental concentrations in the San Luis Drain which have averaged about 350 ppb. Based on the individual acute values for chinook salmon, the calculated margins of safety for selenate and selenite in freshwater were 383 and 276, respectively. However, the margin of safety for selenium as a 6:1 mixture of selenate and selenite in freshwater was 145. Safety margins less than 100 indicate high potential for adverse effects on aquatic resources, thus, the concentrations of selenate and selenite found in the San Luis Drain -- when tested individually or in a 6:1 mixture in freshwater -- do not seem to pose a threat to the health and well-being of chinook salmon from a waterborne route of exposure. However, the toxicity of these selenium forms to fish may change in the presence of other waterborne metal contaminants found in subsurface irrigation drain water or if consumed in selenium-laden food organisms via a dietary route of exposure.

Preliminary results from the 120-day chronic toxicity test indicate that waterborne exposure of young chinook salmon to nominal concentrations of selenium as high as 140 ppb had no effect on their survival or growth; however, complete interpretation of the data must await analysis of selenium concentrations in exposure water and tissue samples, and analysis of fish behavior measurements.

Work Remaining

A manuscript combining the results of two sets of water quality studies with acute toxicity information gained from this work unit and other research efforts has completed NFCR-Columbia and SJVDP in-house review and is currently being reviewed by the FWS editor. The results of the chronic toxicity study with the swimup life stage of chinook salmon exposed to a mixture of selenate and selenite (6:1 ratio) are currently being statistically analyzed; the analytical chemistry results of selenium measurements in fish tissue are expected in late FY89.

Following completion of these analyses, a manuscript will be prepared on the results of the chronic toxicity study.

Table 1. Acute toxicity (LC50s in ppm; 95% confidence intervals in parentheses) of selenite and selenate to different life stages of chinook salmon in soft water.

Chemical	Life stage or weight (g)	Exposure period (hr) 24	96
Selenite	eyed egg	>560	>560
	alevin	202	104
		(151-270)	(87-124)
	0.31	100	27.3
		(84-118)	(19.2-38.7)
	0.46	65.8	13.1
		(0.5-85.6)	(10.8-16.0)
Selenate	eyed egg	>1000	>1000
	alevin	>320	>320
	0.31	511	114
		(381-686)	(85-154)

Table 2. Acute toxicity (LC50s in ppm; 95% confidence intervals in parentheses) of selenite and selenate to different stocks of chinook salmon from California tested in a reconstituted water quality simulating dilution of the San Luis Drain in a freshwater receiving water. One stock of chinook salmon tested was from the Merced River Fish Facility (MR) and the other was from Iron Gate Salmon and Steelhead Hatchery (IG). Also given is the pooled (P) acute toxicity values for the combined data from the two stocks of chinook salmon.

Chemical	Stock	Mean weight (g)	24 hour	96 hour
Selenite	MR	0.5	46.1 (38.2-55.6)	14.8 (11.4-19.1)
	IG	0.7	57.0 (48.3-67.1)	13.0 (8.6-19.5)
	P		46.9 (38.2-57.5)	13.8 (10.8-17.6)
Selenate	MR	0.5	464 (360-600)	121 (86-171)
	IG	0.7	510 (361-721)	100 (74-135)
	P		475 (421-537)	115 (94-141)

ACUTE AND CHRONIC EXPOSURES OF SALMON TO SELENIUM IN BRACKISH WATER

Introduction

Originally the San Luis Drain was planned to terminate at Chipps Island in the western Sacramento-San Joaquin Delta, but federal budget restrictions halted construction of the drain in 1975 at the site of the flow-regulating Kesterson Reservoir. In 1979, the San Joaquin Valley Drainage Program recommended completion of the San Luis Drain to the Delta which would directly deliver irrigation return flows to a brackish-water receiving water. The Delta already receives irrigation return water from the northern portion of the federal service area which drains into the middle reaches of the San Joaquin River.

Entry of elevated concentrations of selenium into the Delta could potentially cause adverse effects on salmonids. Toxicological impacts on salmonids would occur primarily in the pre-smolt life stage when advanced fry are inhabiting the Delta prior to smoltification and seaward migration.

The present research was undertaken to determine the effects on chinook salmon of waterborne exposure to selenium in water qualities which simulate potential environmental conditions in brackish water. Toxicity studies are to be conducted with various forms of selenium individually and in various mixtures to simulate reported environmental ratios.

Methods

The advanced fry life stage (2 g) of fall-run chinook salmon was used in one chronic toxicity study to determine the lethal and sublethal effects of a 120-day exposure to waterborne selenium. The forms of selenium tested were sodium selenate and sodium selenite, and the environmental ratios of selenate:selenite tested was 6:1. The chronic toxicity test was conducted in standardized test waters that simulated cation and anion concentrations (minus trace elements) in standardized San Luis Drain water diluted 10-fold in a standardized brackish-water receiving water (about 1.2 ppthousand salinity). Actual drainwater was not used in toxicity studies because its water quality varies due to location and time of year.

The water quality of the exposure water was pH 7.6, $_2$38 ppm sulfate, and 0.6 ppthousand salinity for the first 30 days while for the remainder of the study it was pH 7.4, 291 ppm sulfate, and 1.2 ppthousand salinity. This reconstituted water quality was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with Instant Ocean sea salts and small amounts of various other salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate. The chronic toxicity test was conducted in an intermittent-flow proportional diluter which delivered 1 liter of reconstitiuted water to each exposure aquaria at 15 minute intervals. Six duplicated nominal concentrations of selenium were tested (0, 17, 35, 70, 140, and 280 ppb). Throughout the study, mortality was recorded daily, fish behavior checklist evaluated weekly, water samples collected weekly for measurement of eight water quality parameters, water samples collected every 15 days for measurement of selenium concentrations, and fish samples collected for measurement of selenium concentrations, fish length and weight measurements, video taping of fish behavior, and measurement of fish swimming performance were accomplished at 30-day intervals. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 15 fish from each treatment following the 120-day exposure.

Progress

Acute toxicity tests with two stocks of young chinook salmon from California tested in a reconstituted brackish water revealed no difference in their sensitivity to selenite and selenate (Table 1). The hazard potential of selenite and selenate to indigenous salmon populations was estimated by comparing the acute toxicity values (96-hour LC50 value; concentration that kills 50% of the test animals in a 96-hour time period) with the expected environmental concentrations in the San Luis Drain which have averaged about 350 ppb. Based on the individual acute values for chinook salmon, the calculated margins of safety for selenate and selenite in brackish water were 479 and 468, respectively. However, the margin of safety for selenium as a 6:1 mixture of selenate and selenite in brackish water was 220. Safety margins less than 100 indicate a high potential for adverse effects on aquatic resources, thus, the concentrations of selenate and selenite found in the San Luis Drain -- when tested individually or in a 6:1 mixture in brackish water -- do not seem to pose a threat to the health and well-being of chinook salmon from a waterborne route of exposure. However, the toxicity of these selenium forms to fish may change in the presence of other waterborne metal contaminants found in subsurface irrigation drain water or if consumed in selenium-laden food organisms via a dietary route of exposure.

Preliminary results from the 120-day chronic toxicity test indicate that waterborne exposure of young chinook salmon to 280 ppb of selenium caused significant reductions in growth of fish after 90 days of exposure and 100% mortality after 100 days of exposure. Complete interpretation of the data must await analysis of selenium concentrations in exposure water and fish tissue and analysis of fish behavior measurements.

Work remaining

A manuscript combining the results of two sets of water quality studies with acute toxicity information gained from this work unit and other research efforts has completed NFCR-Columbia and SJVDP in-house review and is currently being reviewed by the FWS editor. The results of the chronic toxicity study with the advanced fry life stage of chinook salmon exposed to a mixture of selenate and selenite (6:1 ratio) are currently being statistically analyzed; the analytical chemistry results of selenium measurement in fish tissue are expected in late FY89. Following completion of these analyses, a manuscript will be prepared in the results of the chronic toxicity study.

Table 1. Acute toxicity (LC50s in ppm; 95% confidence intervals in parentheses) of selenite and selenate to different stocks of chinook salmon from California in a reconstituted water quality simulating dilution of the San Luis Drain in a brackish-water receiving water. One stock of chinook salmon tested was from the Merced River Fish Facility (MR) and the other was from Iron Gate Salmon and Steelhead Hatchery (IG). Also given is the pooled (P) acute toxicity values for the combined data from the two stocks of chinook salmon.

Chemical	Stock	Mean weight (g)	24 hour	96 hour
Selenite	MR	1.6	56.6 (39.6-80.7)	23.1 (16.6-32.1)
	IG	1.6	75.9 (63.8-90.2)	23.4 (17.2-31.8)
	P		65.6 (52.3-82.3)	23.4 (18.8-29.1)
Selenate	MR	1.6	>600	180 (136-239)
	IG	1.6	316 (236-424)	134 (94-192)
	P		484 (374-626)	149 (116-191)

ACUTE AND CHRONIC EXPOSURES OF SALMON TO SAN LUIS DRAIN CONTAMINANTS IN FRESHWATER

Introduction

Elevated concentrations of several metals were found at 14 sites in the San Joaquin Valley including Kesterson Reservoir, the San Luis Drain collector lines, and a number of drainage canals between Mendota and Gustine, CA. Even though selenium has been identified as the most obvious contaminant of concern in these water samples, significant metal concentrations of boron, chromium, iron, lead, molybdenum, vanadium, and zinc were also found in the samples. Contamination of aquatic habitats, i.e. wetlands, sloughs, and rivers, with elevated concentrations of metals could cause adverse effects on fish and wildlife. Metal-contaminated subsurface drainage water already enters freshwater habitats of the Grasslands area, located south and adjacent to Kesterson, by way of large canals, drains, and small ditches. This water leaves the Grasslands through Mud Slough, which serves as the primary drainage system, and in turn, drains into the San Joaquin River. Subsurface seepage from wetlands adjacent to the San Joaquin River such as Kesterson Reservoir is also another route of entry of contaminants to the river.

Entry of elevated concentrations of metals into the San Joaquin River could potentially cause adverse effects on salmonids. Toxicological impacts on salmonids would occur primarily in the early life stages when fry migrate down the San Joaquin River from spawning areas in tributaries.

The present research was undertaken to determine the effects on chinook salmon of waterborne exposure to metals in water qualities which simulate potential environmental conditions in freshwater.

Methods

The swimup life stage (0.5 g) of fall-run chinook salmon was used in one chronic toxicity study to determine the lethal and sublethal effects of a 120-day exposure to a waterborne mixture of metals in the environmental ratios found in the San Luis Drain. The mixtures of metals tested included arsenate, boron, cadmium, chromate, copper, ferric iron, lead, manganese, mercury, molybdenum, nickel, selenate, selenite, silver, vanadium, and zinc. The chronic toxicity test was conducted in standardized test waters that simulated the major cation and anion concentrations (minus trace elements) in standardized San Luis Drain water diluted 10-fold in a standardized fresh-water receiving water. Actual drainwater was not used in toxicity studies because its water quality varies with location and time of year.

The water quality of the exposure water was pH 7.8, 211 ppm hardness, 108 ppm alkalinity as HCO_3, 46 ppm calcium, 24 ppm magnesium, 70 ppm sodium, 60 ppm chloride, and 186 ppm sulfate. This reconstituted water quality was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with various salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate. The chronic toxicity test was conducted in an intermittent-flow proportional diluter which delivered 1 liter of reconstituted water to each exposure aquaria at 15 minute intervals. Six duplicate nominal concentrations of the metal mixture were tested (Table 1). Throughout the study, mortality was recorded daily, fish behavior checklist evaluated weekly, water samples collected weekly for measurement of eight water quality parameters, water samples collected every 15 days for measurement of selenium concentrations, and fish samples collected for measurement of selenium concentrations, fish length and weight measurements, video taping of fish behavior, and measurement of fish swimming performance were accomplished at 30-day intervals. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 15 fish from each treatment following the 120-day exposure.

Progress

The acute toxicity of a mixture of 13 metals (arsenate, boron, cadmium, chromate, copper, lead, manganese, mercury, molybdenum, nickel, silver, vanadium, and zinc) in their environmental ratios found in standardized San Luis Drain water was equal in its toxicity to young chinook salmon tested in freshwater as when the mixture contained selenate and selenite. This result suggests that the waterborne toxicity of the mixture of metals was not dependent on the presence of selenate or selenite but rather on the joint toxicity of the other metal components of the mixture. Based on an evaluation of toxic units (a toxic unit is the ratio of the LC50 concentration of a metal in a mixture to the LC50 concentration of a metal tested individually -- LC50 value is the concentration that kills 50% of the test animals in a specific time period), the major toxic component of the metal mixture was copper (toxic unit 0.488-0.510) followed closely by cadmium and boron (Table 2). Selenate and selenite had relatively low toxic unit values thus indicating their small contribution to the waterborne toxicity of the mixture of metals which was tested in their environmental ratios found in standardized San Luis Drain water.

Both the 13-metal mixture with and without selenate and selenite showed additive toxicity characteristics rather than antagonistic or synergistic characteristics. Both metal mixtures were also about twice as toxic to young salmon tested in freshwater as they were to older salmon tested in brackish water.

The hazard potential of the metal mixture with and without selenate and selenite to salmon populations was estimated by comparing the acute toxicity values with the expected environmental concentrations in the San Luis Drain. Based on the joint acute toxicity of the environmental mixture of metals, the calculated margin of safety for the mixture without selenium was 53.2 whereas for the mixture with selenium it was 55.6. Safety margins less than 100 indicate a high potential for adverse effects on aquatic resources, thus, it is clear from the results of these acute toxicity tests that the mixture of metals found in the San Luis Drain pose a "waterborne threat" to the health and well-being of young chinook salmon in freshwater regardless of whether selenium is present in the mixture or not.

Preliminary results from the 120-day chronic toxicity study indicate that waterborne exposure to nominal concentrations of the mixture of 16 metals in the high concentration treatment (Table 1) for 120 days had no effect on survival or growth of chinook salmon; however, complete interpretation of the data must await analysis of concentrations of selected metals in exposure water and tissue samples, and analysis of fish behavior measurements. The high treatment concentration of the metal mixture tested in the chronic toxicity study was about 10 times lower than the 96-hour LC50 value identified in acute toxicity tests.

Work remaining

A manuscript combining the results of two sets of water quality studies with acute toxicity information gained from this work unit and other research efforts is currently in preparation -- the Materials and Methods sections and data tables have been completed. The results of the chronic toxicity study with the swimup life stage of chinook salmon exposed to a mixture of 16 metals/metalloids are currently being statistically analyzed; the analytical chemistry results of metal measurements in fish tissue are expected in late FY89. Following completion of these analyses, a manuscript will be prepared on the results of the chronic toxicity study.

Table 1. Nominal concentrations (ppb) of a mixture of metals used in a 120-day chronic toxicity exposure of young chinook salmon and reared in a reconstituted fresh water.

	Treatment (ppb)					
Metal	1	2	3	4	5	6
Arsenate	0	0.03	0.06	0.12	0.24	0.48
Boron	0	325	650	1300	2600	5200
Cadmium	0	0.025	0.05	0.1	0.2	0.4
Chromate	0	0.875	1.75	3.5	7	14
Copper	0	0.125	0.25	0.5	1	2
Iron (III)	0	2.5	5	10	20	40
Lead	0	0.05	0.1	0.2	0.4	0.8
Manganese	0	0.5	1	2	4	8
Mercury	0	0.004	0.008	0.016	0.032	0.064
Molybdenum	0	1.875	3.75	7.5	15	30
Nickel	0	0.5	1	2	4	8
Selenate	0	7.5	15	30	60	120
Selenite	0	1.25	2.5	5	10	20
Silver	0	0.025	0.05	0.1	0.2	0.4
Vanadium	0	0.75	1.5	3	6	12
Zinc	0	0.375	0.75	1.5	3	6

Table 2. Nominal concentrations (ppb) and (in parentheses) the individual toxic units of each component in SLD contaminant mixtures that produced 50% mortality in a 96-hour period in chinook salmon (0.9 g) tested in freshwater and the acute joint toxicities of each mixture.

Constituent	Mix I	Mix II
Mercury	0.85 (0.008)	0.89 (0.009)
Cadmium	5.3 (0.205)	5.6 (0.216)
Silver	5.3 (<0.001)	5.6 (<0.001)
Arsenate	6.4 (<0.001)	6.7 (<0.001)
Lead [a]	10.6	11.1
Copper	26.6 (0.488)	27.8 (0.510)
Zinc	79.8 (0.063)	83.4 (0.066)
Manganese [a]	106	111
Nickel [a]	106	111
Vanadium	160 (0.010)	167 (0.010)
Chromium	186 (0.002)	195 (0.002)
Molybdenum [b]	399 (<0.001)	417 (<0.001)
Boron [b]	69160 (0.095)	72280 (0.100)
Selenite	N.P. [c]	278 (0.019)
Selenate	N.P.	1670 (0.014)
Ratio (LC50: concentration in SLD)	53.2	55.6
Joint Toxicity		
Toxic Units	0.871	0.946
Additive Index	0.148	0.057
(range)	(-0.639-0.165)	(-0.659-0.733)

[a] Toxic unit value not determined.
[b] Individual toxicities taken from Hamilton and Buhl (in prep.)
[c] Not present.

ACUTE AND CHRONIC EXPOSURES OF SALMON TO SAN LUIS DRAIN CONTAMINANTS IN BRACKISH WATER

Introduction

Agricultural wastewater from irrigation acitivites in the San Joaquin Valley of California has been reported to contain high concentrations of boron, chromium, iron, lead, molybdenum, vanadium, and zinc. These elevated concentrations of metals were found in a number of drainage canals between Mendota and Gustine, including Kesterson Reservoir, and the San Luis Drain collector lines. Subsurface drainage water already enters fresh-water habitats of the Grasslands area, located south and adjacent to Kesterson, by way of large canals, drains, and small ditches. Entry of metal-laden irrigation return flows into the San Joaquin River and subsequent transport to the Sacramento-San Joaquin Delta could cause adverse effects on fish and wildlife. Likewise, completion of the San Luis Drain from Kesterson Reservoir to Chipps Island in the Delta, which is a drainwater management option, could directly deposit metal-laden water to aquatic habitats in the Delta.

Entry of elevated concentrations of metals into the Delta could potentially cause adverse effects on salmonids. Toxicological impacts on salmonids would occur primarily in the pre-smolt life stages when fry are inhabiting the Delta prior to smoltification and seaward migration.

The present research was undertaken to determine the effects on chinook salmon of waterborne exposure to metals in water qualities which simulate potential environmental conditions in brackish water.

Methods

The advanced fry life stage (2 g) of fall-run chinook salmon was used in one chronic toxicity study to determine the lethal and sublethal effects of a 120-day exposure to a waterborne mixture of 16 metals in the environmental ratios found in the San Luis Drain. The mixture of metals tested included arsenate, boron, cadmium, chromate, copper, ferric iron, lead, manganese, mercury, molybdenum, nickel, selenate, selenite, silver, vanadium, and zinc. The chronic toxicity test was conducted in standardized test waters that simulated cation and anion concentrations (minus trace elements) in standardized San Luis Drain water diluted 10-fold in a standardized brackish-water receiving water (about 1.2 ppthousand salinity). Actual drainwater was not used in toxicity studies because its water quality consistency varies due to location and time of year. The water quality of the exposure water was pH 7.5, 238 ppm sulfate, and 0.6 ppthousansd salinity for the first 30 days while for the remainder of the study it was pH 7.3, 291 ppm sulfate, and 1.2 ppthousand salinity. This reconstituted water quality was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with Instant Ocean sea salts and small amounts of various other salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate.

The chronic toxicity test was conducted in an intermittent-flow proportional diluter which delivered 1 liter of reconstituted water to each exposure aquaria at 15 minute intervals. Six duplicate nominal concentrations of the metal mixture were tested (Table 1). Throughout the study, mortality was recorded daily, fish behavior checklist evaluated weekly, water samples collected weekly for measurement of eight water quality parameters, water samples collected every 15 days for measurement of selenium concentrations, and fish samples collected for measurement of selenium concentrations, fish length and weight measurements, video taping of fish behavior, and measurement of fish swimming performance were accomplished at 30-day intervals. A 10-day seawater challenge in 28 ppthousand seawater was conducted with 15 fish from each treatment following the 120-day exposure.

Progress

The acute toxicity of a mixture of 13 metals (arsenate, boron, cadmium, chromate, copper, lead, manganese, mercury, molybdenum, nickel, silver, vanadium, and zinc) in their environmental ratios found in standardized San Luis Drain water was equal in its toxicity to juvenile chinook salmon tested in brackish water as when the mixture contained selenate and selenite. This result suggests that the waterborne toxicity of the mixture of metals was not dependent on the presence of selenate or selenite but rather on the joint toxicity of the other metal components of the mixture. Based on an evaluation of toxic units (a toxic unit is the ratio of the LC50 concentration of a metal in a mixture to the LC50 concentration of a metal tested individually -- LC50 value is the concentration that kills 50% of the test animals in a specific time period), the major toxic component of the metal mixture was copper (toxic unit 0.992-1.140) followed closely by boron, cadmium, and mercury (Table 2). Selenate and selenite had relatively low toxic unit values, thus indicating their small contribution to the waterborne toxicity of the mixture of metals which were tested in their environmental ratios found in standardized San Luis Drain water.

Both the 13-metal mixture with and without selenate and selenite showed antagonistic characteristics and, consequently, was less toxic to chinook salmon than it would have been expected to based on the individual toxicity of the metal components of the mixture. Both metal mixtures were also about half as toxic to juvenile salmon tested in brackish water as they were to young salmon tested in freshwater.

The hazard potential of the metal mixture with and without selenate and selenite to salmon populations was estimated by comparing the acute toxicity values with the expected environmental concentrations in the San Luis Drain. Based on the joint acute toxicity of the environmental mixture of metals, the calculated margin of safety for the mixture without selenium was 119 whereas for the mixture with selenium it was 137. Safety margins less than 100 indicate a high potential for adverse effects on aquatic resources, thus, the results of these acute toxicity tests indicate that the margin of safety for the mixture of metals found in the San Luis Drain is close to the criteria value for posing a "waterborne threat" to the health and well-being of juvenile chinook salmon in brackish water.

Preliminary results from the 120-day chronic toxicity study indicate that waterborne exposure to nominal concentrations of the mixture of 16 metals in the high concentration treatment (Table 1) for 120 days had no effect on survival or growth of chinook salmon; however, complete interpretation of the data must await analysis of concentrations of selected metals in exposure water and tissue samples, and analysis of fish behavior measurements. The high treatment concentration of the metal mixture tested in the chronic toxicity study was about 10 times lower than the 96-hour LC50 value identified in acute toxicity tests.

Work remaining

A manuscript combining the results of two sets of water quality studies with acute toxicity information gained from this work unit and other research efforts is currently in preparation -- the Materials and Methods sections and data tables have been completed. The results of the chronic toxicity study with the advanced fry life stage of chinook salmon exposed to a mixture of 16 metals/metalloids are currently being statistically analyzed; the analytical chemistry results of metal measurements in fish tissue are expected in late FY89. Following completion of these analyses, a manuscript will be prepared on the results of the chronic toxicity study.

Table 1. Nominal concentrations (ppb) of a mixture of metals used in a 120-day chronic toxicity exposure of young chinook salmon and reared in a reconstituted fresh water.

Metal	Treatment (ppb) 1	2	3	4	5	6
Arsenate	0	0.06	0.12	0.24	0.48	0.96
Boron	0	650	1300	2600	5200	10400
Cadmium	0	0.05	0.1	0.2	0.4	0.8
Chromate	0	1.75	3.5	7	14	28
Copper	0	0.25	0.5	1	2	4
Iron (III)	0	5	10	20	40	80
Lead	0	0.1	0.2	0.4	0.8	1.6
Manganese	0	1	2	4	8	16
Mercury	0	0.008	0.016	0.032	0.064	0.128
Molybdenum	0	3.75	7.5	15	30	60
Nickel	0	1	2	4	8	16
Selenate	0	15	30	60	120	240
Selenite	0	2.5	5	10	20	40
Silver	0	0.05	0.1	0.2	0.4	0.8
Vanadium	0	1.5	3	6	12	24
Zinc	0	0.75	1.5	3	6	12

Table 2. Nominal concentrations (ppb) and (in parentheses) the individual toxic units of each component in SLD contaminant mixtures that produced 50% mortality in a 96-hour period in chinook salmon (1.8 g) tested in brackish water and the acute joint toxicities of each mixture.

Constituent	Mix I	Mix II
Mercury	1.90 (0.113)	2.19 (0.130)
Cadmium	11.9 (0.208)	13.7 (0.240)
Silver	11.9 (<0.001)	13.7 (<0.001)
Arsenate	14.3 (<0.001)	16.4 (<0.001)
Lead [a]	23.8	27.4
Copper	59.5 (0.992)	68.4 (1.140)
Zinc	179 (0.062)	205 (0.071)
Manganese [a]	238	274
Nickel [a]	238	274
Vanadium	357 (0.022)	410 (0.025)
Chromium	417 (0.003)	479 (0.003)
Molybdenum [b]	893 (<0.001)	1026 (<0.001)
Boron [b]	154700 (0.258)	177840 (0.297)
Selenite	N.P.	684 (0.030)
Selenate	N.P.	4100 (0.023)
Ratio (LC50: concentration in SLD)	119	137
Joint Toxicity		
Toxic Units	1.658	1.959
Additive Index	-0.658	-0.959
(range)	(-1.584—-0.065)	(-1.823—-0.362)

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[a] Toxic unit value not determined.

[b] Individual toxicities taken from Hamilton and Buhl (in prep.)

[c] Not present.

CHRONIC DIETARY EXPOSURE OF SALMON TO SELENIUM IN FRESHWATER OR BRACKISH WATER

Introduction

Following discovery of selenium contamination in the San Luis Drain and its terminus, Kesterson Reservoir, field investigations showed that selenium was being bioaccumulated in aquatic food-chain organisms at concentrations toxic to consumer animals. Elevated selenium concentrations were found in fish from the Helm Canal in the Grasslands, located south and adjacent to Kesterson. Wetland habitats of the Grasslands receive subsurface drain water from large canals, drains, and small ditches. Elevated selenium concentrations were also found in fish from Mud Slough which is the primary drainage system for the Grasslands; Mud Slough drains into the San Joaquin River.

Discharge of water containing elevated concentrations of selenium in food-chain organisms to the San Joaquin River or to the Sacramento-San Joaquin Delta could potentially cause adverse impacts on salmonids from a dietary route of exposure. Toxicological impacts on salmonids would occur primarily in the early life stages when fry migrate down the San Joaquin River from spawning areas in tributaries. Additional impacts could occur on advanced fry inhabiting nursery areas in the Delta where they undergo parr-smolt transformation and migrate to the sea.

The present research was undertaken to determine the effects on chinook salmon of dietary exposure to selenium in water qualities which simulate potential environmental conditions in freshwater and brackish water.

Methods

Two chronic toxicity studies were conducted with chinook salmon exposed to selenium in the diet in 1986 to determine the effects on survival, growth, and whole-body burdens of selenium. In both studies three Oregon Moist Pellet diets were used; typically, 40% of the dry ingredients in the diet is salmon meal. In our diets we replaced half the salmon meal with fish meal made from ground, freeze-dried mosquitofish prior to mixing dry ingredients together and preparing the final product. The control diet contained uncontaminated mosquitofish meal, one series of dietary selenium exposures contained mosquitofish meal from fish collected from the San Luis Drain (termed SLD diet), and a second series of dietary selenium exposures contained uncontaminated mosquitofish spiked with selenomethionine (termed SEM diet).

In both toxicity studies, the concentrations of selenium tested in each of two selenium-containing diets were 3.2, 5.3, 9.6, 18.2, and 35.4 ppm dry weight; the control diet contained 1.0 ppm selenium dry weight. Each dietary treatment was fed to 100 fish in two replicate aquaria. Mortality was recorded daily, growth was measured on two 10-fish subgroups within each replicate aquaria at 30-day intervals, and fish were sampled at 30-day intervals and water at 15-day intervals for selenium determinations.

The first study was conducted for 90 days and started with the swimup life stage and a reconstituted water quality simulating dilution of standardized San Luis Drain water (minus trace elements) in a standardized fresh-water receiving water. Actual drain water was not used in toxicity studies because its water quality varies with location and time of year. The water quality of the test water was pH 7.7, 210 ppm hardness, 109 ppm alkalinity as HCO_3, 45 ppm calcium, 23 ppm magnesium, 70 ppm sodium, 59 ppm chloride, and 186 ppm sulfate. This reconstituted water quality was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with various salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate. This study is termed the fresh-water study.

The second study was conducted for 120 days and started with 70 mm/2 g-sized fish and a reconstituted water quality similar to the first study except a brackish-water receiving water was used. The water quality of the test water was pH 7.4, 235 ppm sulfate, and 0.6 ppthousand salinity for the first 30 days while for the remainder of the study it was pH 6.9, 291 ppm sulfate, and 1.2 ppthousand salinity. This reconstituted water quality was created as in the first study except that the majority of salts added were Instant Ocean sea salts. This study is termed the brackish-water study.

Analysis of selenium concentrations in water and fish from both studies was accomplished on digested samples with hydride generation atomic absorption. Water samples were digested by a persulfate technique and tissue samples by a nitric acid wet digestion and magnesium nitrate dry ash technique.

Progress

Analysis of concentrations of 22 elements in the diets revealed that the major toxic component in the SLD diet was selenium but concentrations of boron, chromium, and strontium were also elevated compared to concentrations in the control and SEM diets. Concentrations of selenium in water were below the limit of quantitation (0.59-1.45 ppb) in all dietary selenium exposures in both chronic toxicity studies. Selenium was not directly added to water in these experiments but was indirectly added to water from excretory products from fish and dissolution of diet; however, these sources of selenium were minimized by daily cleaning and siphoning out of feces and uneaten food from exposure aquaria.

Fresh-water study: Survival was reduced by exposure to 9.6 ppm selenium for 90 days in both dietary exposures (Figure 1). Growth, as measured by length and weight, was reduced by exposure to ≥ 5.3 ppm selenium for 90 days in both dietary exposures (Figures 2 and 3). Whole-body burdens of selenium were increased in fish fed ≥ 3.2 ppm selenium in both selenium-containing diets at all sampling periods (Figure 4). A strong dose-response relation between concentration of dietary selenium and survival, growth, and whole-body burdens of selenium was apparent after 90 days of exposure to both selenium diets.

Brackish-water study: Survival was not affected in fish fed either selenium-containing diet for 120 days. In the SLD diet study, fish length was reduced significantly by exposure to ≥ 18.2 ppm selenium for 120 days (Figure 5) whereas fish weight was reduced significantly by exposure to ≥ 9.6 ppm selenium (Figure 6). In the SEM diet study, fish length and weight were both reduced significantly by exposure to 35.4 ppm selenium for 120 days (Figures 5 and 6). Survival of fish in a 10-day seawater challenge test following 120 days of dietary exposure was reduced in fish fed 35.4 ppm selenium in both selenium diets (Figure 7). Whole-body burdens of selenium were increased in fish fed ≥ 3.2 ppm selenium in both selenium-containing diets at all sampling periods (Figure 8). A strong dose-response relation between the concentration of dietary selenium and growth was apparent after 120 days of exposure to the SLD diet but not to the SEM diet. Whole-body burdens of selenium in both studies were also correlated with exposure concentration of dietary selenium.

General conclusions: In both studies, fish fed the SLD diets had higher whole-body residues of selenium than those fed the SEM diets. This difference in selenium uptake between diet types may have been due to the presence of organic forms of selenium other than selenomethionine in the SLD diet, enhanced uptake protein-bound organoselenium in the SLD diet (compared to free selenomethionine in the SEM diet), or the presence of elevated concentrations of boron, chromium, and strontium in the SLD diet.

Work remaining

A manuscript describing these two chronic toxicity studies (fresh-water study and brackish-water study) has been completed and revised according to suggestions received from three NFCR-Columbia and one SJVDP in-house reviewers. The revised manuscript is currently being reviewed by the Fish and Wildlife Service fisheries editor.

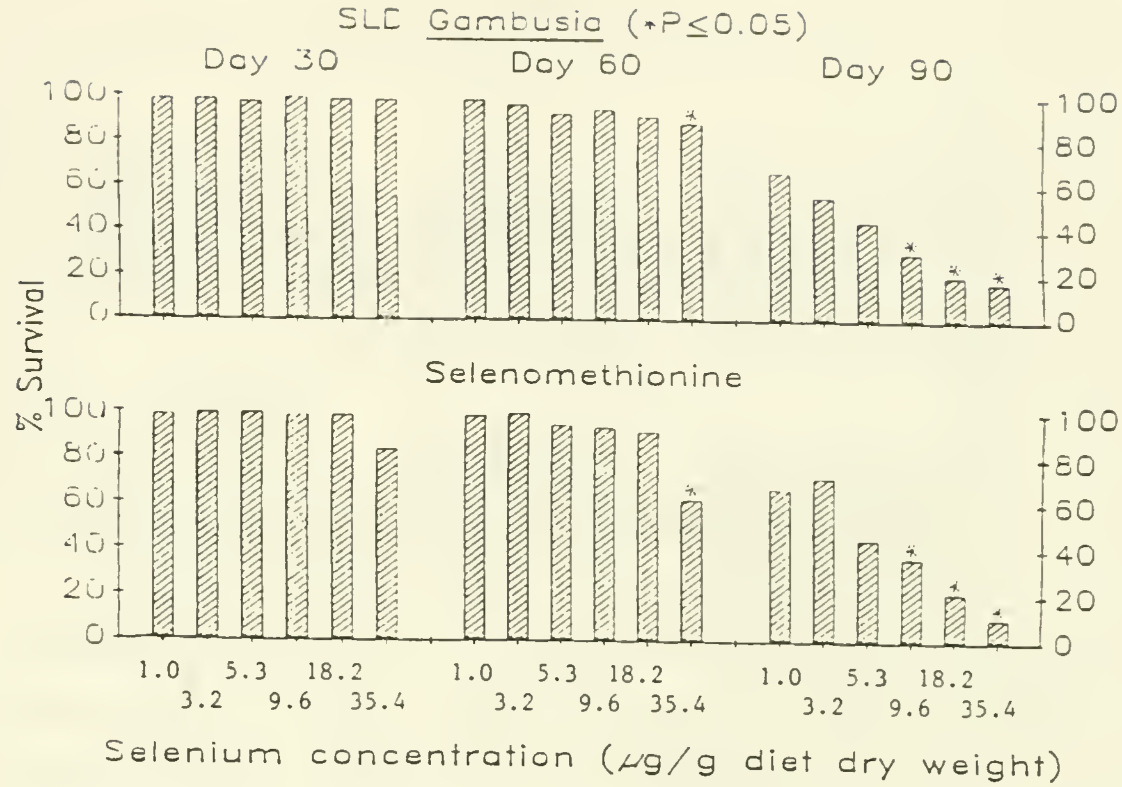

Figure 1. Survival of chinook salmon exposed to selenium in the diet and in a reconstituted water quality simulating a freshwater situation.

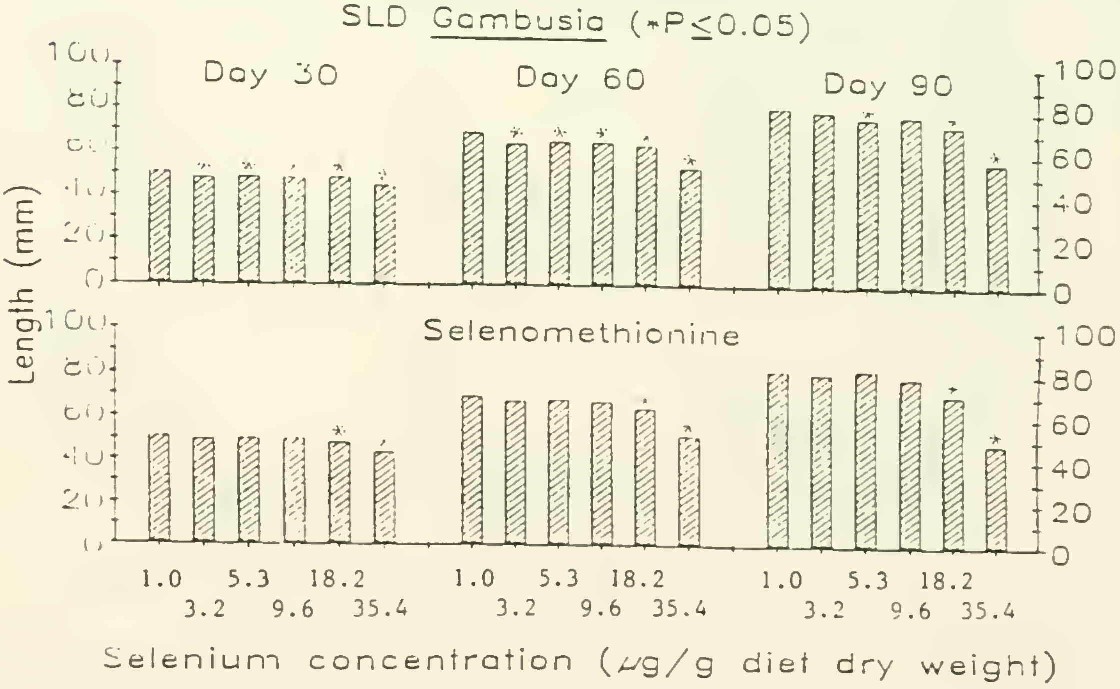

Figure 2. Total length of chinook salmon exposed to selenium in the diet and in a reconstituted water quality simulating a freshwater situation.

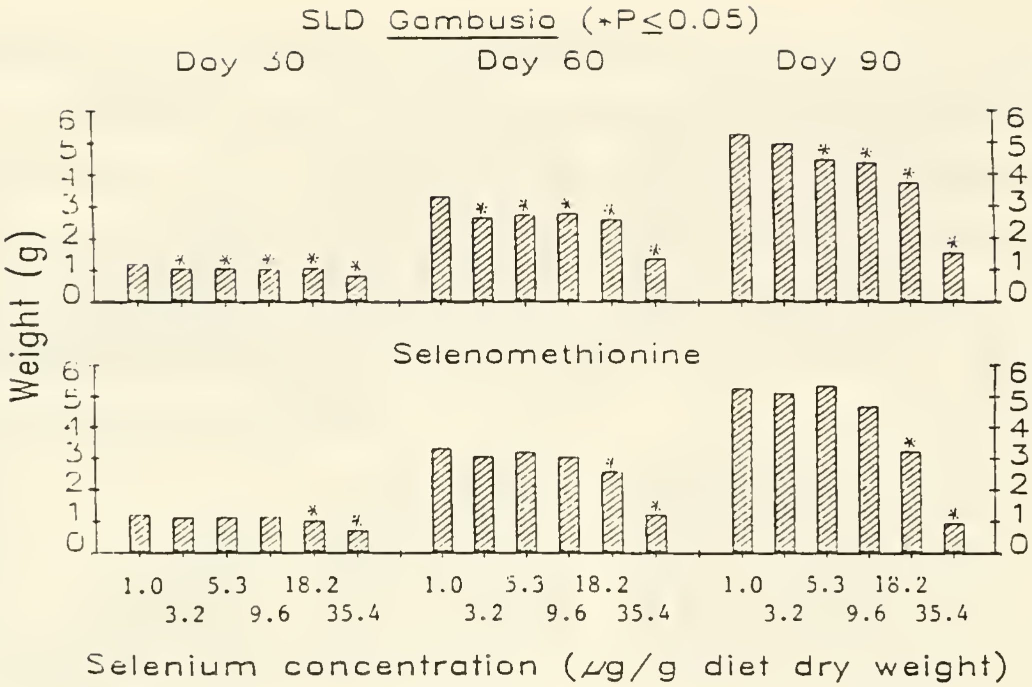

Figure 3. Weight of chinook salmon exposed to selenium in the diet and in a reconstituted water quality simulating a freshwater situation.

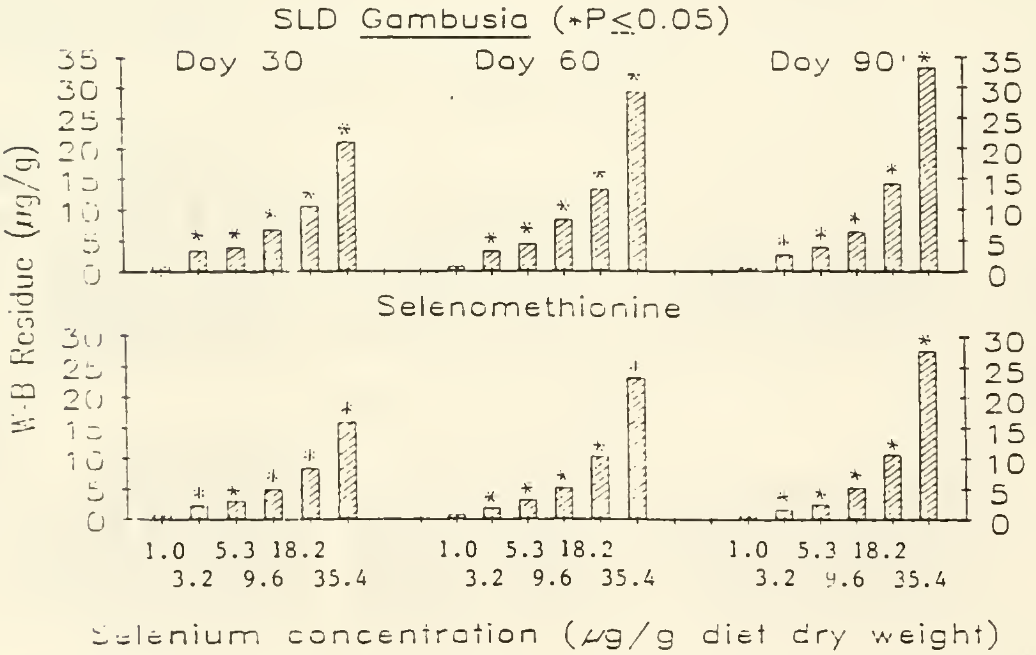

Figure 4. Whole-body burdens of selenium in chinook salmon exposed to the element in the diet and in a reconstituted water quality simulating a freshwater situation.

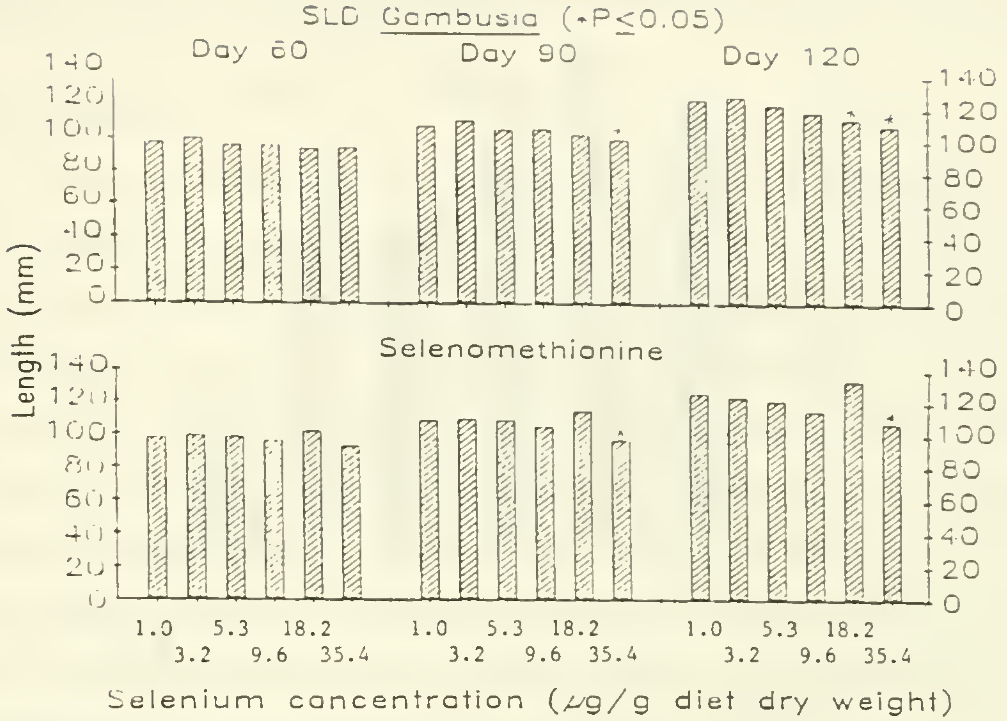

Figure 5. Total length of chinook salmon exposed to selenium in the diet and in a reconstituted water quality simulating a brackishwater situation.

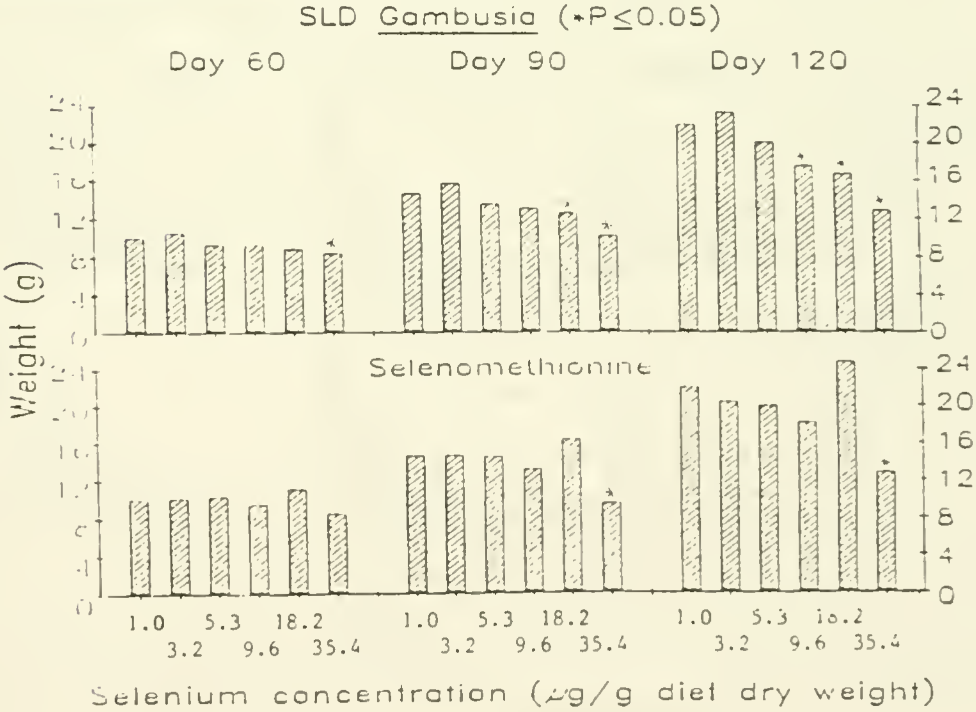

Figure 6. Weight of chinook salmon exposed to selenium in the diet and in a reconstituted water quality simulating a brackishwater situation.

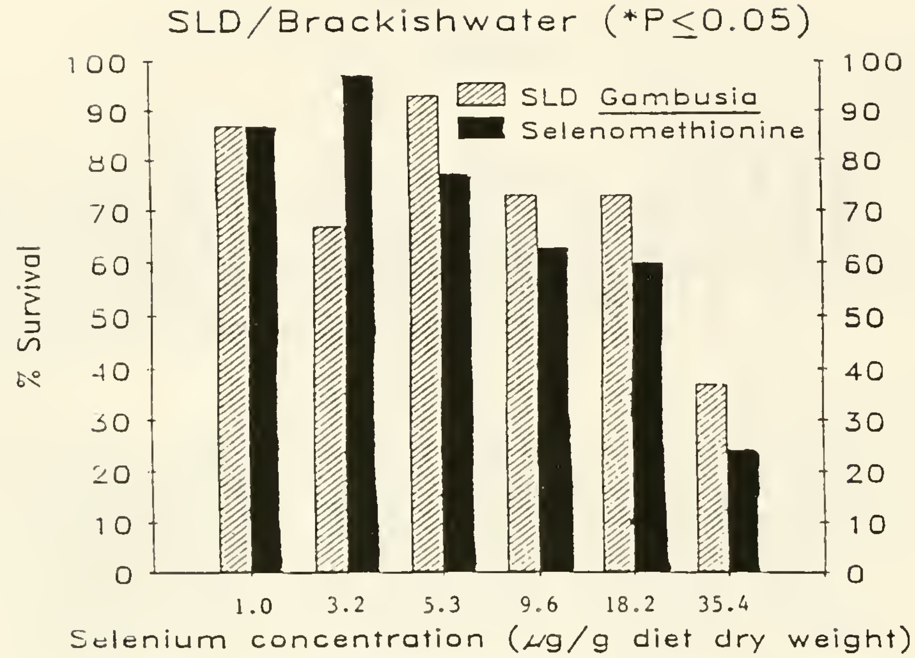

Figure 7. Survival of chinook salmon from a 10-day seawater challenge following 120 days of exposure to selenium in the diet and in a reconstituted water quality simulating a brackishwater situation.

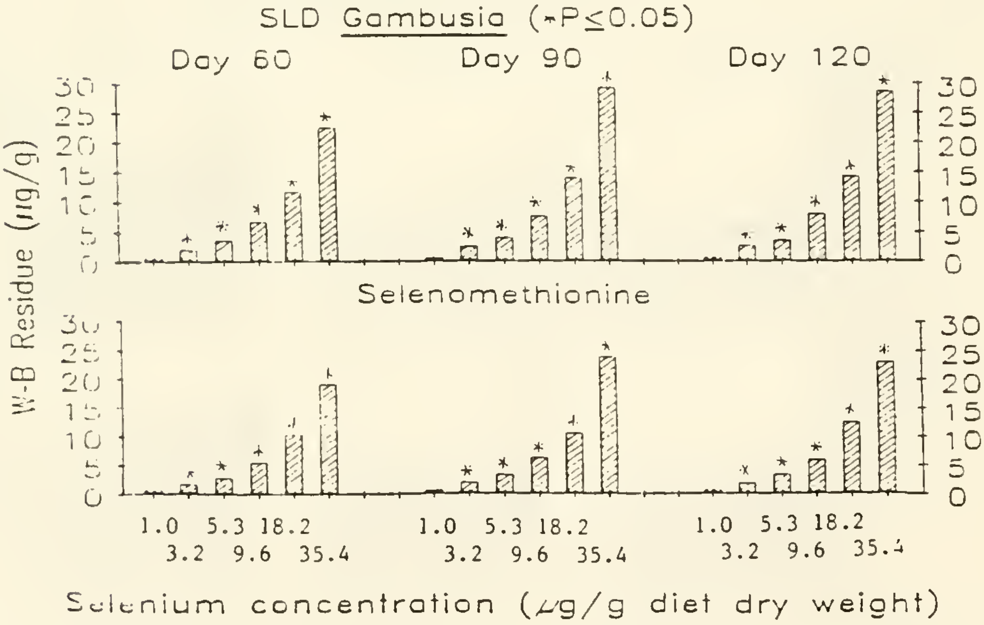

Figure 8. Whole-body burdens of selenium in chinook salmon exposed to selenium in the diet and a reconstituted water quality simulating a freshwater situation.

CHRONIC DIETARY AND WATERBORNE EXPOSURE OF SALMON TO SELENIUM IN FRESHWATER OR BRACKISH WATER

Introduction

Field investigations in areas surrounding Kesterson Reservoir, the terminus of the San Luis Drain, have shown that elevated concentrations of selenium are present in both water and food-chain organisms. The source of the selenium is from large canals, drains, and small ditches which deposit subsurface drainage water in the Grasslands, located south and adjacent to Kesterson Reservoir. The primary drainage system for the Grasslands is Mud Slough which is a tributary of the San Joaquin River. Entry of water and food-organisms containing elevated concentrations of selenium into the San Joaquin River and subsequent transport to the Sacramento-San Joaquin Delta could cause adverse effects on fish and wildlife. Likewise, completion of the San Luis Drain from Kesterson Reservoir to Chipps Island in the Delta, which is a drainwater management option, could directly deliver selenium-laden water and food-chain organisms to aquatic habitats in the Delta. The presence of selenium in both water and food-organisms allows two routes of uptake that could affect fish and wildlife resources.

Salmonids in the San Joaquin River of the Delta could be exposed to selenium in water and food-organisms if entry of selenium-laden water to these two aquatic habitats is allowed to occur. Toxicological impacts on salmonids would occur primarily in the early life stages when fry migrate down the San Joaquin

River from spawning areas in tributaries. Additional impacts could occur on advanced fry inhabiting nursery areas in the Delta where they undergo parr-smolt transformation and migrate to the sea.

The present research was undertaken to determine the effects on chinook salmon of dietary exposure to selenium in water qualities which simulate potential environmental conditions in freshwater and brackish water.

Methods

Two chronic toxicity studies were conducted with chinook salmon exposed to selenium in both water and diet in 1987 to determine the effects on survival, growth, and whole-body burdens of selenium. In the first study, the swimup life stage was exposed for 120 days to 12 duplicated treatments; nominal concentrations of selenium that comprised the treatments were 0, 17, and 70 ppb (6:1 ratio of selenate:selenite) in water and 0, 4, 9, and 18 ppm (dry weight) in the form of selenomethionine in the diet. The matrix below shows the 12 treatment combinations that were tested in a water quality simulating dilution of standardized San Luis Drain water (minus trace elements) in a standardized fresh-water receiving water.

		Water		
		0	17	70
D	0	1	5	9
i	4	2	6	10
e	9	3	7	11
t	18	4	8	12

Actual drain water was not used in toxicity studies because its water quality consistency varies due to location and time of year. The water quality of the exposure water was pH 7.6, 211 ppm hardness, 110 ppm alkalinity as HCO_3, 46 ppm calcium, 24 ppm magnesium, 70 ppm sodium, 60 ppm chloride, and 186 ppm sulfate. This reconstituted water quality was created by mixing high purity water from a water softener-reverse osmosis-deionizer system with various salts such as calcium sulfate, magnesium sulfate, sodium chloride, and sodium bicarbonate. This study is termed the fresh-water study.

In the second study, 70 mm/2 g-sized fish were exposed for 120 days to 12 duplicated treatments; nominal concentrations of selenium that comprised the treatments were 0, 35, and 140 ppb (6:1 ratio of selenate:selenite) in water and 0, 9, 18, and 36 ppm (dry weight) in the form of selenomethionine in the diet. The matrix below shows the 12 treatment combinations that were tested in a water quality similar to the first study except a brackish-water receiving water was used.

		Water		
		0	35	170
Diet	0	1	5	9
	9	2	6	10
	18	3	7	11
	36	4	8	12

The water quality of the exposure water was pH 7.4, 246 ppm sulfate, and 0.6 ppthousand salinity for the first 30 days while for the remainder of the study it was pH 7.0, 287 ppm sulfate, and 1.2 ppthousand salinity. This reconstituted water quality was created as in the first study except that the majority of salts added were Instant Ocean sea salts. This study is termed the brackish-water study.

Progress

Fresh-water study: The control and selenium-laden diets were analyzed for concentrations of boron, cadmium, selenium, and zinc. The control and treatment diets contained similar concentrations of boron (2.2-3.2 ppm), cadmium (0.04 ppm), and zinc (175-185 ppm); however, the control diet contained 0.87 ppm selenium (dry weight) and the selenium diets contained 5.0, 9.2, and 17.4 ppm selenium.

The measured waterborne concentrations of selenium were 1 (control), 14, and 62 ug/L compared with nominal concentrations of 0, 17, and 70 ug/L, respectively. Concentrations of selenium in unfiltered aquaria exposure water were nearly identical with concentrations of selenium measured in water samples filtered through a glass fiber filter and then a 0.4 um polycarbonate filter.

Whole-body burdens of selenium in chinook salmon in the fresh-water study seemed to be highly influenced by dietary selenium exposure and only slightly influenced by waterborne selenium exposure (Table 1). At each exposure sample period and within a specific dietary selenium exposure, whole-body burdens of selenium were only slightly increased with increasing waterborne selenium exposure concentrations. However, at each exposure sample period and within a specific waterborne selenium exposure concentration, whole-body burdens of selenium were greatly increased with increasing dietary selenium exposure. Moreover, whole-body burdens of selenium in chinook salmon seemed to be due almost exclusively to dietary exposure to selenium.

Brackish-water study: The selenium concentrations in the control and selenium-laden diets were 0.87 (control), 9.2, 17.4, and 35.4 ppm (dry weight). The measured waterborne concentrations of selenium were 0.1 (control), 32, and 112 ppb compared with nominal concentrations of 0, 35, and 140 ug/L, respectively.

Whole-body burdens of selenium in chinook salmon in the brackish-water study followed a trend nearly identical to that observed in the fresh-water study (Table 2). Whole-body burdens of selenium were highly influenced by dietary selenium exposure and only slightly influenced by waterborne selenium exposure; in fact, whole-body burdens of selenium in chinook salmon seemed to be due almost exclusively to dietary exposure to selenium.

Work remaining

Data on mortality and growth (fish length and weight) from both the fresh-water and brackish-water studies is currently being statistically analyzed. A manuscript describing the results of these two studies will be prepared in FY89.

Table 1. Mean and standard error (in parentheses) of whole-body concentrations of selenium (ppm dry weight) in chinook salmon exposed to waterborne and dietary selenium for 120 days in a water quality simulating dilution of standardized San Luis Drain water in a standardized freshwater.

Days of exposure	Waterborne exposure (ppb)	Dietary exposure (ppm dry weight)			
		0.9	5.0	9.2	17.4
30	1	1.26 (0.04)	3.13 (0.16)	5.75 (0.01)	9.20 (0.48)
	14	1.42 (0.14)	3.43 (0.32)	5.06 (0.04)	8.30 (0.37)
	62	1.67 (0.03)	3.66 (0.01)	5.57 (0.17)	9.11 (0.83)
60	1	1.17 (0.05)	3.62 (0.05)	5.77 (0.09)	11.65 (0.45)
	14	1.51 (0.12)	5.11 (0.74)	5.72 (0.16)	11.35 (0.75)
	62	2.11 (0.41)	4.06 (0.69)	6.69 (0.18)	12.30 (0.80)
90	1	1.14 (0.11)	3.33 (0.02)	5.53 (0.25)	10.02 (0.58)
	14	1.18 (0)	3.67 (0.30)	4.68 (1.66)	12.50 (0.80)
	62	1.64 (0.18)	3.79 (0.29)	5.04 (0.20)	9.09 (3.01)
120	1	0.94 (0.01)	3.06 (0.18)	5.41 (0.16)	11.45 (0.95)
	14	1.02 (0.02)	3.50 (0.36)	5.44 (0.26)	11.35 (0.05)
	62	1.29 (0.13)	3.42 (0.45)	6.22 (0.50)	11.85 (0.95)

Table 2. Mean of whole-body concentrations of selenium (ppm dry weight) in chinook salmon exposed to waterborne and dietary selenium for 120 days in a water quality simulating dilution of standardized San Luis Drain water in a standardized brackish water.

Days of exposure	Waterborne exposure (ppb)	Dietary exposure (ppm dry weight)			
		0.9	9.2	17.4	35.4
30	0.1	0.78	2.29	4.38	7.35
	32	0.92	2.53	4.53	7.26
	112	0.99	2.93	4.44	7.70
60	0.1	0.85	3.01	4.78	9.00
	32	0.82	2.67	4.51	6.53
	112	0.97	3.60	5.96	9.10
90	0.1	0.60	3.45	7.70	11.8
	32	0.70	3.74	6.36	12.8
	112	1.08	3.60	6.19	12.9
120	0.1	0.77	3.56	6.13	13.9
	32	0.77	3.40	6.29	14.0
	112	1.13	4.32	6.35	13.2

TOLERANCE OF JUVENILE FALL CHINOOK SALMON TO SELENIUM EXPOSURE FROM WATER AND THE FOOD CHAIN: IMPACTS ON SMOLTIFICATION AND EARLY MARINE SURVIVAL

Introduction

The fall chinook salmon (*Oncorhynchus tshawytscha*) native to the Central Valley of California are a valuable renewable natural resource that could be adversely impacted if selenium (Se) from irrigation return flows were to contaminate surface waters such as the Sacramento River or the San Joaquin River. To properly evaluate disposal alternatives for San Joaquin Valley drainwater, information is needed to determine the maximum safe exposure of resident fish species to waterborne inorganic Se as well as to dietary organoselenium. In FY-86 and FY-87 we established the safe limits of organoselenium intake via the diet for smolting fall chinook salmon. In FY-88, our objective was to determine the safe limit for exposure of chinook smolts to inorganic Se in the water supply.

The safe limit, or tolerance limit, is the highest level of Se that cannot be shown to be unsafe. To adequately protect migrating fall chinook, we defined the tolerance limit for chinook salmon smolts as the highest concentration of inorganic or organic Se that does not interfere either with the normal development of smoltification during freshwater rearing, nor with the ability to adapt to seawater, nor with the ability to continue to grow and develop normally at salinities of 30 parts per thousand (ppt). We assessed effects on the parr-smolt transformation and on early marine survival by histological examination of gill, kidney and other tissues, by seawater challenge/plasma sodium tests and evaluation of gill sodium/potassium adenosine triphosphatase (Na+/K+ ATPase) development, by a volitional outmigration test, and by freshwater and seawater survival and growth.

Methods

We obtained chinook salmon from Washington State Department of Fisheries' Minter Creek Hatchery. One day after we transported the fish to MFS, we treated them with 166 parts per million (ppm) formalin for 1h to control ectoparasites. We then placed lots of 300 at 2.3 g per fish in 12 2.5-ft. diameter, 70-gallon capacity, circular tanks at a flow rate of 1 gallon per minute (gpm) fresh water at a loading density of less than 0.05 lbs per gallon and less than 3.5 lbs per gpm. We fed them a 2% active terramycin-medicated Oregon Moist Pellet (OMP) diet at 3% body weight per day for 10 days to control subclinical bacterial infection, and then began exposing them to Se in the water supply. We fed the fish OMP at standard hatchery feeding rates, but not exceeding 3% body weight per day throughout the experiment.

Due to an unexpected event, stocks did not receive the prescribed one month acclimation period prior to beginning the Se exposure. The original fish that we had obtained, and that we had quarantined and observed, treated medically, acclimated to our facility for 6 weeks, grown from less than 1 g to 3.5 g each, and dispensed into experimental tanks, were all killed on May 5, when the fresh water supply was unexpectedly interrupted in the week prior to the start of the experiments. We replaced them with the same stocks, but they had not been uniformly groomed, as had the original fish, nor was there time to prepare them as we normally do prior to their anticipated transformation from parr to smolts. Although fish were not of uniform size, they had been prepared for release from the hatchery. Therefore, we eliminated the acclimation, and accelerated the disease treatment over the 12 days immediately preceeding selenium exposure.

We exposed duplicate groups of fall chinook salmon to 0 (control), 35, 70, 140, 280, and 560 ug/L Se. The test solutions contained a 6:1 ratio of the +6 and +4 oxidation states of Se, derived from sodium selenate (Na2SeO4) and sodium selenite (Na2SeO3). We used a diluter as described by Garten (1980) to deliver the appropriate concentrations, and we conducted the Se exposure for 7 weeks from May 17 to July 4, when we started converting the fish to seawater.

We took histology samples at 0, 3 and 6 weeks after beginning the Se exposure, and then 2 months after conversion to seawater. Gill, pseudobranch, thymus, liver, kidney, digestive tract, brain, olfactory nerve, and spinal cord were examined. We fixed the tissues in Bouin's fixative, embedded them in paraffin and stained them with hematoxylin and eosin and May-Grunwald Giemsa. We also stained selected tissues using the direct fluorescent antibody technique (FAT) and/or Gram stain for the detection of bacterial kidney disease.

We monitored fresh water growth by individually weighing and measuring 20 branded fish from each group before and after the 7-week Se-exposure period. Not all branded fish were recovered, due to mortality, accidental sampling, or difficulty in reading brands after 7 weeks. Mortality was recorded daily.

We evaluated effects of waterborne Se on osmoregulatory ability by conducting weekly 24-h seawater challenge/plasma sodium tests on each test and control group during the exposure period. To conduct a test, we transferred 10 fish from freshwater directly to full-strength seawater (30 ppt) for 24 h, counted mortalities, took blood samples from survivors, and determined plasma sodium concentrations by flame photometry.

Smoltification is also characterized by a progressive increase in the activity of gill Na+/K+ ATPase, an enzyme which is part of the chloride cell salt pump system required by anadromous salmonids to osmoregulate in seawater. We assessed effects on the normal ATPase development pattern by taking gill tissue specimens from 10-fish samples from each test and control group at weekly intervals during the Se exposure period, and also after fish had been in seawater for 4 weeks. Gills were stored frozen and analysed for Na+/K+ ATPase activity according to procedures described by Zaugg (1982). Results are expressed as micrograms of inorganic phosphate released from adenosine triphosphate per milligram of protein in the gill tissue preparation per hour of incubation.

Smoltification culminates in downstream migration and entry into the sea, where the young fish must survive, grow, and develop in order to contribute to the fishery. We measured effects of inorganic Se on migratory behavior by freeze-branding 50-fish lots from each test and control group and transporting them to the U.S. Fish and Wildlife Service's Quilcene National Fish Hatchery for a volitional out-migration test. We placed three notched dividers in a standard 8-ft by 80-ft hatchery raceway at 20-ft intervals to form 4 pools, each 8 inches shallower than the preceeding one. The first pool was 32 inches deep. Water flow was set at 250 gpm and the marked fish placed and confined with screens in the upstream end of the modified raceway. After a 4-day acclimation period, the restraining screen was removed, allowing the branded fish freedom to migrate. Each day, fish were netted from the last downstream pool, identified by brand, and scored as migrants. Water flow was checked each day and maintained at 250 gpm.

We evaluated seawater growth and survival of the smolts for 3 months following Se exposure. Sixty fish from each test and control group were anaesthetized and individually weighed and measured at the end of the Se exposure in fresh water, and then at monthly intervals following conversion to seawater.

Progress

Histopathology: Not completed. Samples are being processed.

Freshwater Mortality: Mortality during the 7 weeks of Se exposure ranged from a minimum of 0.7% at 280 ug/L Se to a maximum of 4.0% at 560 ug/L. Mortality was highest in the group exposed to the highest concentration of Se tested, but none was significantly higher than controls (Table 1).

Freshwater Growth: There was no trend toward reduced growth or size at the higher concentrations of Se tested. The final lengths and weights of control fish were the smallest, and the largest fish after 7 weeks were those exposed to 560 ug/L Se (Table 2). Percent increase in either length or weight did not correlate with concentration of Se tested.

Seawater Challenge: The parr-smolt transformation is normally accompanied by a progressively increased ability of juvenile salmon to maintain physiologically normal levels of plasma sodium (less than 170 meq/L) when challenged with 30 ppt seawater. Normally, pre-smolted salmon respond to a 24-h seawater challenge test with high percent mortality, and high plasma Na+ concentration in survivors. As fish develop osmoregulatory competence, both percent mortality, and mean plasma Na+ concentration in survivors, decline.

The fish that we obtained on May 7 did not smolt normally in the time they were present at MFS. Percent mortality after seawater challenge was lowest (0% mortality) in subsamples of stocks tested on May 11, the earliest date for which data is available, and it increased thereafter (Table 3). But plasma Na+ concentrations were high on that date (192 meq/L), and at no time during the subsequent 8 weeks did they decline to the levels expected of fully functional smolts. Overall percent mortality after 24-h seawater challenge was less than 10% on May 31, but rose to greater than 70% on June 21, and after that it remained high, suggesting that parr-reversion had taken place.

Increased percent mortality after seawater challenge began to rise one week earlier than in the controls even at the lowest concentration of Se tested, when on June 13 all groups except controls showed a marked increase in percent mortality after 24-h seawater challenge. Controls rose only from 10% to 15%, while the next lowest increase in mortality was from 15% to 35% in the group exposed to 140 ug/L Se. In the test started June 20, fall chinook exposed to 280 ug/L Se for 34 days before 24-h seawater challenge had a significantly higher plasma Na+ concentration than controls, but this value has little physiological significance, since none of the exposure groups was osmoregulating normally by that date.

Gill Na+/K+ ATPase Activity: All groups had their highest gill Na+/K+ ATPase activities on 6/3, the first day of sampling, after 17 days on selenium (Table 4). ATPase activities declined after that until the end of the selenium exposure period. These results suggest a loss in osmoregulatory ability, as do those from the seawater challenge experiments. Despite the apparent parr-reversion, all groups were physiologically able to adapt to seawater if the conversion was gradual. Thirty-five days after seawater entry, all groups had high ATPase activities, with no indication of reduced activity as a result of selenium exposure.

Migratory Behavior: Most fish migrated in the first week, and there was no reduced percent migration at any concentration of Se tested. After one week, cumulative percent migrations ranged from 50% to 63%, with 55% of controls migrating (Table 5). After four weeks cumulative migrations ranged from 53% to 66% and 59% of the controls had migrated.

Seawater Survival: Only about 65% of the fish converted to full-strength seawater (30 ppt) on 7/8 survived for 3 months, with seawater survival of all exposure groups about the same as controls (Table 6). Survival at one and two month intervals was also comparable to controls.

Seawater Growth: There was fairly uniform growth in all groups in seawater (Table 7). By the end of three months, none was substantially smaller than controls.

Body Composition: Not completed. Samples are being analysed.

Summary: We saw no clear indication of the toxicity of waterborne selenium to smolts of fall chinook salmon at any level tested. Fresh water mortality and growth were comparable to controls at selenium concentrations up to 560 ug/L. Since the fish were past the peak of smoltification when we tested them, we were unable to test the effect of waterborne selenium on the process of smoltification. However, we saw no effects on ability to osmoregulate, as measured by periodic 24-h seawater challenge experiments and measurements of gill Na+/K+ ATPase activity. We observed no decrease in migratory tendency, even at 560 ug/L Se, and seawater survival and growth were comparable to controls in all effluent exposure levels tested.

Work Remaining:

Tissue samples collected during the study are being analyzed for abnormalities associated with selenium exposure and for contaminant burdens. Once completed, this information will be evaluated and incorporated in the final report prepared during FY'89.

References

1. Garten, Ronald R. 1980. A simple continuous-flow toxicant delivery system. Water Research, 14: 227-230.

2. Zaugg, W.S. 1982. A simplified preparation for adenosine triphosphatase determination in gill tissue. Canadian Journal of Fisheries and Aquatic Sciences, 39: 215-217.

Table 1. Percent mortality of fall chinook salmon during 7-week exposure to inorganic selenium in the freshwater supply.

Se concentration (μg/L)	Percent Mortality
0 (control)	1.5
35	3.2
70	1.3
140	1.3
280	0.7
560	4.0

Table 2. Growth of fall chinook salmon exposed to the indicated concentration of inorganic selenium in the water supply for 7 weeks during freshwater rearing. There was no reduction in growth at any level of selenium exposure.

Se concentration (μg/L)	Starting Mean Length (MM)	Final Mean Length (MM)	Percent Increase	Starting Mean Length (g)	Final Mean Length (g)	Percent Increase
0 (control)	59.9	84.6	41	2.63	6.97	165
35	66.3	89.5	35	3.57	8.66	143
70	61.6	88.8	44	2.84	7.79	174
140	63.6	88.3	39	3.09	7.80	152
280	64.1	90.8	42	3.25	8.51	162
560	67.1	91.3	36	3.59	8.76	144

Table 3. Plasma Na+ concentration and percent mortality of selenium-exposed fall chinook salmon subjected to a 24-h seawater challenge test. "Day" indicates the number of days before or after the beginning of Se exposure, on which 24-h seawater challenge was begun.

Se concentration ($\mu g/L$)	Date Day	5/10 -7	5/17 0	5/30 13	6/5 19	6/13 27	6/20 34	6/16 40	7/4 48
	Plasma Na+ concentration (meq/L)								
0 (control)		192	206	187	204	206	221	213	212
35		192	206	197	200	200	225	217	211
70		192	206	196	201	207	220	199	214
140		192	206	196	204	208	230	205	206
280		192	206	194	194	210	257*	210	212
560		192	206	202	199	209	230	205	214
	Percent Mortality								
0 (control)		0	55	10	10	15	85	55	85
35		0	55	30	20	45	85	35	60
70		0	55	5	25	65	85	85	75
140		0	55	0	15	35	75	65	90
280		0	55	10	15	50	60	75	60
560		0	55	0	15	52	80	65	70

* statistically significant, P = 0.05, Dunnett's test.

Table 4. Gill Na+/K+ ATPase activity (μg Pi·mg prot^{-1}·h^{-1}) (SD) at selected intervals of 20-fish lots of juvenile fall chinook salmon exposed to different concentrations of waterborne selenium for the indicated number of days. Fish were removed form selenium exposure on 7/4 and gradually converted to full-strength seawater. Fish sampled on 8/12 had been in full-strength seawater for 35 days.

Date (1988)	6/3	6/17	7/1	8/12
Day	17	31	45	--
Se concentration ($\mu g/L$)	Mean ATPase Activity (SD)			
0 (control)	13.47	9.48	6.52	30.29
	(4.0148)	(1.9506)	(1.3710)	(5.5113)
35	15.27	9.19	7.27	36.13
	(2.9745)	(2.0055)	(1.5574)	(8.0481)
70	13.71	9.67	6.81	30.15
	(3.7374)	(2.0347)	(2.3493)	(7.0983)
140	13.32	8.75	7.52	34.72
	(3.8021)	(1.9631)	(1.9887)	(13.2299)
280	15.95	8.22	7.10	31.30
	(4.8587)	(1.9800)	(2.2396)	(8.1353)
560	16.73	9.56	7.36	29.53
	(2.1460)	(2.0803)	(1.9720)	(7.0690)

Table 5. Effect of inorganic selenium in the freshwater supply on migratory behavior of fall chinook salmon exposed for 48 days prior to release. "Days" indicates the number of days after release. No significant differences occurred.

Days	7	14	21	28
Se concentration (μg/L)	Cumulative Percent Migration			
0 (control)	55	56	58	59
35	52	56	56	56
80	50	53	53	56
140	63	64	66	66
280	55	56	56	57
560	56	57	58	59

Table 6. Effect of waterborne selenium on the early marine survival of juvenile fall chinook salmon exposed for 48 days prior to seawater entry.

Date (1988) Months in SW ($30^{o}/oo$)	8/11 1	9/6 2	10/6 3
Se concentration (μg/L)	Percent Survival		
0 (control)	91	85	64
35	95	88	68
70	97	88	73
140	91	80	67
280	93	73	53
560	92	86	68

Table 7. Effect of 48 day exposure to waterborne selenium on subsequent seawater growth of juvenile fall chinook salmon.

Date (1988)	7/7	8/11		9/6		10/5	
Months in seawater	0	1		2		3	
Se concentration (µg/L)	L(mm)	L(mm)	% inc	L(mm)	% inc	L(mm)	% inc
0 (control)	90.8	105.8	16.5	115.3	26.9	126.2	39.0
35	91.3	106.5	16.7	116.4	27.5	126.7	38.8
70	90.5	105.9	17.0	115.7	27.9	127.6	41.1
140	89.7	105.4	17.5	116.4	29.7	126.0	40.4
280	91.8	105.7	15.2	115.9	26.3	126.9	38.2
560	91.5	107.0	16.9	115.9	27.8	124.8	36.5
	Wt(g)	Wt(g)	% inc	Wt(g)	% inc	Wt(g)	% inc
0	8.8	14.3	63.1	19.9	127	25.8	194
35	9.0	14.4	60.3	20.4	127	26.2	191
70	8.6	14.0	63.1	20.2	135	27.2	217
140	8.6	14.0	62.9	20.1	133	26.1	203
280	8.9	13.8	55.2	20.0	125	26.2	195
560	9.0	14.6	62.5	21.1	135	25.3	182

ACUTE TOXICITY OF SELENIUM OXIDATION FORMS TO THE INVERTEBRATES *DAPHNIA MAGNA* AND *CHIRONOMUS RIPARIUS*, AND THE ALGAE *SELENASTRUM CAPRICORNUTUM*

Introduction

The U.S. Bureau of Reclamation has initiated a comprehensive study program to provide information on the potential environmental impact of contaminants in irrigation drain water. The information will also be used by the California State Water Resources Control Board for regulating discharges of drain water into state waters.

Collection of San Joaquin Valley drain water into a series of evaporation ponds has created a contamination problem at Kesterson Reservoir. In response to this problem, NFCRC proposed to assess drain water contaminant impacts on aquatic resources in the San Joaquin Valley. The impact of selenium on associated fisheries and aquatic resources has not be adequately addressed. The goal of this research is to determine the effects of selenium associated with agricultural drain water on fish and aquatic invertebrates. Information is needed on the sublethal effects of both water and dietary selenium exposure to aquatic organisms.

The objective for this study was to determine the acute toxicity of several forms of selenium to the cladoceran, *D. magna* and midge *C. riparius*, and the green algae *Selenastrum capricornutum*. Additional NFCRC studies address selenium bioaccumulation and chronic toxicity to *D. magna* and *C. riparius*.

Methods

Four 48-h acute toxicity tests were conducted with both invertebrates: 1) sodium selenate, 2) sodium selenite, 3) a 6:1 mixture of sodium selenate to sodium selenite (as selenium) and 4) seleno-L-methionine. Two different dilution waters were used in testing: 1) a reconstituted freshwater representative of the San Joaquin Valley region (hardness = 134 mg/L as $CaCO_3$, alkalinity = 60-65 mg/L as $CaCO_3$, sulfate = 72 mg/L) and 2) standard ASTM soft water (hardness = 40-48 mg/L as $CaCO_3$, alkalinity = 30-35 mg/L as $CaCO_3$, sulfate = 54 mg/L). Tests were initiated with animals < 24 h old. Mortality was recorded at 24 and 48 hours. Death was defined as a cessation of all visible signs of mobility during a 5-s observation with the unaided eye. The 48-h LC50 was calculated by either probit, moving average, or Spearman-Karber methods.

Progress

All acute toxicity test with *D. magna* and *C. riparius* have been completed. *D. magna* are more sensitive to the acute toxic effects of inorganic selenium compared to *C. riparius* (Table 1) The selenate form of selenium tended to be less toxic than either the selenite form or the 6:1 mixture of selenate to selenite. Seleno-L-methionine was extremely toxic to *D. magna*. Daphnids experienced 50-70% mortality at seleno-L-methionine concentrations of about 4 ug/L as Se, however no clear dose response was demonstrated.

C. riparius was acutely affected at seleno-L-methionine concentrations about 1000 fold higher than D. magna. The reason for daphnids not exhibiting a dose response to the organic form of selenium remains unclear. Perhaps a substantial portion of the seleno-L-methionine volatilized in the midge exposure chambers. Selenium concentrations were only measured at the beginning of the exposures. D. magna tended to be more sensitive selenium in reconstituted ASTM soft water compared to reconstituted freshwater representative of the San Joaquin Valley region. No such trend was evident with C. riparius (Table 1).

Waterborne inorganic Se is generally toxic to invertebrates at levels greater than the recommended freshwater Criterion Continuous Concentration of 5 µg Se/L. While the toxicity of inorganic Se to freshwater invertebrates has been thoroughly investigated, little data are available on the toxicity of organoselenium compounds. We observed selenomethionine to be acutely lethal to D. magna at about 4 µg Se/L. Assuming an application factor of 0.04, the calculated Maximum Acceptable Toxicant Concentration for selenomethionine would be about 0.16 µg Se/L (see the following report: "Chronic toxicity of selenium to the invertebrates Daphnia magna and Chironomus riparius"). Within the Kesterson National Wildlife Refuge the concentration of dissolved organoselenium forms reportedly represents up to 27% of the total Se. Additional data are needed on the toxicity and fate of naturally occurring organoselenium compounds in fresh water before the hazard of Se in the aquatic environment can be adequately addressed.

Work Remaining

Toxicity tests with algae Selenastrum capricornutum were postponed because of reduced SJVDP funding. If funding is sufficient next fiscal year, studies will be initiated to address bioaccumulation and toxicity. It would also be appropriate to investigate why there is such a large difference in the acute lethal response of the two invertebrate species to the organic form of selenium. The acute toxicity data for daphnids and midge have been incorporated into a manuscript that is currently in NFCRC review. This manuscript will be submitted to Aquatic Toxicology in FY 89.

Table 1. Selenium 48-h LC50 values (mg/L) for *D. magna* and *C. riparius*.

	Species	
	D. magna	*C. riparius*
Reconstituted freshwater		
Sodium selenate	4.07(3.22-5.00)	16.2[c]
Sodium selenite	3.02(2.48-3.76)	7.95(5.21-14.5)
Inorganic selenium mixture[a]	2.62(2.02-3.39)	9.34(6.04-12.3)
Seleno-L-methionine	b	5.78(4.10-7.68)
Reconstituted ASTM soft water		
Sodium selenate	2.56(2.02-3.22)	10.5 (7.91-13.4)
Sodium selenite	0.700[c]	14.6 (12.0-18.2)
Inorganic selenium mixture[a]	1.79(1.39-2.33)	14.3 (12.3-17.0)
Seleno-L-methionine	b	6.88(5.30-8.87)

[a] Mixture of 6:1 sodium selenate to sodium selenite (as Se)
[b] no dose response
[c] no confidence interval could be calculated

CHRONIC TOXICITY OF SELENIUM TO THE INVERTEBRATES *DAPHNIA MAGNA* AND *CHIRONOMUS RIPARIUS*

Introduction

The U.S. Bureau of Reclamation has initiated a comprehensive study program to provide information on the potential environmental impact of contaminants in irrigation drain water. The information will also be used by the California State Water Resources Control Board for regulating discharges of drain water into state waters.

Collection of San Joaquin Valley drain water into a series of evaporation ponds has created a contamination problem at Kesterson Reservoir. In response to this problem, NFCRC proposed to assess drain water contaminant impacts on aquatic resources in the San Joaquin Valley. The impact of selenium on associated fisheries and aquatic resources has not be adequately addressed. The objective for this study was to determine the chronic toxicity of a 6:1 mixture of sodium selenate to sodium selenite (as Se) in a reconstituted freshwater representative of the San Joaquin Valley region.

Life-cycle selenium toxicity tests were conducted with two freshwater invertebrates: the cladoceran, *D. magna* and midge, *C. riparius*. Endpoints monitored included effects of selenium exposure on survival, growth, reproduction or whole body ions of these two species. These studies are part of a series of studies designed to determine the toxicological significance of selenium in agricultural drainwater in the Central Valley of California.

Survival, growth and reprodutive data from these studies were reported in the FY 87 Annual report. Data on the ionoregulatory response of D. magna are summarized in this report.

Methods

D. magna were exposed in separate flow-through bioassays to a 6:1 mixture of sodium selenate to selenite (as Se) in a 21-d life cycle test. The dilution water was a reconstituted freshwater blended to be representative of the San Joaquin Valley region. Appropriate amounts of calcium and magnesium sulfate were added to a 25% dilution of NFCRC well water to achieve the following water quality: hardness 134 mg/L as $CaCO_3$; alkalinity 60-65 mg/L as $CaCO_3$; sulfate 72 mg/L.

Nominal exposure concentrations as total selenium in the 6:1 mixture for the D. magna exposure were 1500, 750, 375, 188, 94 and 0 ug/L. D. magna survival and reproduction were monitored three times per week (i.e. MWF). In addition, daphnids less than 24-h old were collected from the first brood of adults from each exposure beaker for length measurements.

Seven to ten surviving daphnids at the end of the 21-d exposure were sampled from each replicate exposure beaker for whole body ion measurements. The animals were blotted dry, weighed (±0.01 mg), pooled, and then dried to a constant weight at $60^{o}C$. While both wet and dry weight measurements were taken, elemental concentrations have been expressed in terms of dry weight. Instrumental Neutron Activation Analysis (INAA) was carried out at the University of Missouri Research Reactor.

Total Mg, Ca, K, Na, and Cl content of the daphnids was determined after a 1 min pneumatic tube irradiation (flux density = 8×10^{13} $n \cdot cm^{-2}$) and counted for 10 min using a Ge(Li) detector gamma-ray spectrometer system after a 3 min delay. For Se the irradiation, count time and delay was 5 s, 25 s and 15 s, respectively. The following isotopes and energies (KeV) were used: ^{27}Mg (1014), ^{49}Ca (3084), ^{42}K (1525), ^{24}Na (1368), ^{38}Cl (1642), and ^{77}Se (162). Interlaboratory cross check analyses previously conducted at our laboratory indicate an average difference between INAA and hydride generation atomic absorption spectroscopy of 8.6% for Se.

Progress

All chronic toxicity tests have been completed. D. magna reproduction and intrinsic rate of natural increase (r) were reduced at Se concentrations ≥ 348 µg/L. Daphnid adult growth was reduced at Se concentrations ≥ 156 µg/L (Table 1). Whole body Mg, K, and Na in daphnids were not affected by chronic Se exposure, however whole body Ca increased at intermediate Se concentrations. In addition, whole body Cl was reduced at 711 µg Se/L. Daphnids exposed to increasing Se concentrations experienced increased Se accumulation and decreased Se bioconcentration factors (BCF) (Table 2). Daphnids accumulated Se in this waterborne exposure which could adversely affect fish or waterfowl that consume these animals (see the report: "Effects of Selenium on Bluegill Reproduction").

Ionoregulation by fish has been demonstrated to be a sensitive sublethal indicator of exposure to many organic and inorganic contaminants. Freshwater crustacea obtain many ions such as sodium and calcium by active uptake directly from water. Hence, effects of toxicants on the ionoregulatory capacity of invertebrates may also be a sensitive measure of contaminant stress.

In the present study, changes in whole body ions were not sensitive indicators of Se toxicity to D. magna. Perhaps the animals were able to acclimate to elevated Se concentrations during the 21-d exposure. During long-term exposures both fish and invertebrates may compensate physiologically to contaminant exposure and regain net ionoregulatory balance. The only significant effect we observed relative to the controls was an elevation in whole body calcium at intermediate Se concentrations (156-348 µg Se/L). Se reportedly interferes with calcium metabolism of both fish and invertebrates. Additional research is needed to better define the normal range in ion concentrations of D. magna and to determine the time course in changes in ion regulation by invertebrates resulting from exposure to contaminants.

Work Remaining

A manuscript summarizing the results of both the acute and chronic toxicity studies is currently in NFCRC review. The manuscript will be submitted to Aquatic Toxicology in FY 89.

Table 1. Effects of chronic exposure to an inorganic mixture of sodium selenate to sodium selenite (6:1 as selenium) on mean survival, and growth of *D. magna*. Mean values (±SEM) sharing a common letter are not significantly different ($p > 0.05$).

Selenium[1] ug/L	n	Survival (%)	Day first Gravid	Total Young	Young/ AFRD[2]	r[3]	Weight[4] (ug)	Body length newborn (mm)
4	4	92.5(±4.15)a	8.0(±0.35)	1613(±269)a	10.3(±1.61)a	0.451(±0.02)a	881(±35)a	0.731(±0.01)a
85	3	93.3(±5.44)a	9.0	1868(±201)a	12.0(±0.96)a	0.470(±0.01)a	770(±48)ab	0.764(±0.01)b
156	4	100.0 a	8.5(±0.43)	1885(± 76)a	11.8(±0.48)a	0.458(±0.01)a	670(±25)bc	0.772(±0.01)b
348	4	97.5(±2.17)a	11.8(±1.08)	858(±126)b	5.4(±0.79)b	0.402(±0.01)b	567(±36)cd	-
718	3	93.3(±2.72)a	11.0(±1.63)	459(± 66)b	3.0(±0.51)b	0.401(±0.01)b	508(±18)d	-
1410	4	5.0(±5.00)	-	-	-	-	-	-

[1] Total selenium; a 6:1 mixture of sodium selenate to sodium selenite as selenium.
[2] Young produced per surviving adult reproductive day.
[3] Intrinsic rate of natural increase.
[4] Mean individual dry weight/replicate.

Table 2. Effects of chronic selenium exposure to _D_. _magna_ on whole body Mg, Ca, K, Na, Cl (μg/mg), Se (μg/g) and the Bioconcentration Factor (BCF). Mean values (±SEM) sharing a common letter are not significantly different ($p > 0.05$). No samples were analyzed from animals exposed to 1410 μg Se/L.

Se[1]	Mg	Ca	K	Na	Cl	Se	BCF
5	2.18 (±0.17)a	23.5 (±0.85)a	6.26 (±0.54)a	7.05 (±0.50)a	6.93 (±0.80)ab	1.3 (±0.1)a	322 (±24.8)a
85	1.84 (±0.15)a	22.2 (±1.19)a	7.81 (±0.74)a	7.05 (±0.61)a	6.90 (±0.64)ab	21.1 (±1.8)b	248 (±21.4)b
156	1.97 (±0.16)a	28.5 (±0.97)b	7.91 (±0.74)a	7.08 (±0.37)a	7.10 (±0.45)a	14.7 (±0.6)b	94 (±4.1)c
348	2.01 (±0.25)a	27.6 (±1.19)b	6.51 (±0.47)a	6.64 (±0.24)a	6.19 (±0.34)ab	31.7 (±3.0)c	91 (±8.7)c
711	1.58 (±0.04)a	23.3 (±1.49)a	7.31 (±0.49)a	6.09 (±0.44)a	4.83 (±0.28)b	51.1 (±3.0)d	71 (±4.2)c

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[1] Total selenium (μg/L); a 6:1 mixture of sodium selenate to sodium selenite as selenium.

ACUTE AND CHRONIC EFFECTS OF VARIOUS FORMS OF WATERBORNE SELENIUM, SINGLY, AND IN COMBINATION ON JUVENILE STRIPED BASS

Introduction

Suisun Bay has been proposed as a possible receiving area for irrigation discharges from the Central Valley of California. Because of the seleniferious characteristics of the soils in the Central Valley, the irrigation discharges are laden with potentially toxic concentrations of selenium and other contaminants. Suisun Bay serves as habitat for important recreational and commercial species such as striped bass and chinook salmon. However, the impact of contaminated irrigation discharges on the biota of receiving waters has not been adequately assessed. The National Fisheries Contaminant Research Center through an intra-agency agreement with the Bureau of Reclamation conducted studies to determine the toxicity and bioconcentration potential of selenium in striped bass.

Methods

Acute toxicity studies: A series of acute toxicity studies with striped bass were conducted in both a generic brackish water (described later) and standard ASTM soft water (hardness, 40-48 mg/L as $CaCO3$; alkalinity, 30-35 mg/L as $CaCO3$; pH, 7.2-7.6). Striped bass were exposed for 96-hours to sodium selenate, sodium selenite, a 6:1 mixture of sodium selenate to sodium selenite, and seleno-L-methionine. The studies were conducted in 19.6 L glass jars that contained 15 L of exposure water. Treatments were spiked to obtain the desired exposure concentrations and there was a 60% dilution factor between concentrations. Ten fish were exposed to each test concentration and mortality was monitored every 24 hours.

Chronic toxicity studies: A partial chronic toxicity study was conducted for 90 days in a flow through proportional diluter. Sacramento River strain striped bass (about 80 days old) were exposed to a 6:1 mixture of sodium selenate to sodium selenite as selenium. The fish were obtained from the California Department of Fish and Game's Central Valley Fish Hatchery as 1-day old larvae.

The experimental water was a generic brackish water representative of Suisun Bay and was prepared by blending appropriate amounts of Instant Ocean^R with deionized (DI) water to yield a salinity of 1.2 g/L. Sodium bicarbonate was added to the water to increase its alkalinity to 70-75 mg/L as $CaCO_3$. The blended water was stored in 8,327 L polyolefin tanks for delivery by a pressurized system to the diluter. The study was conducted at 18^oC and the fish were fed a commercial diet three times daily.

Sodium selenate (98% pure, 41.8% selenium) and sodium selenite (98% pure, 45.7% selenium) stock solutions were prepared in DI water at concentrations corrected for percent purity and percent selenium in the compounds. The stock solutions were prepared in brown glass containers and metered to the chemical mixing cell of the diluter separately by an automatic pipette. Exposure concentrations of selenium in the 6:1 mixture were 2.7, 1.9, 0.64, 0.32, and 0.16 mg/L for selenate and 0.43, 0.21, 0.11, 0.05, and 0.03 mg/L for selenite, constituting total selenium exposure concentrations of 3.0, 1.5, 0.75, 0.375, and 0.188 mg/L. Fish were also exposed to duplicate controls with no selenium added.

Ten striped bass were placed into each small chamber of the exposure aquaria for growth determinations and 40 fish were placed in each large chamber for tissue residues and behavioral observations. Growth samples (total length and weight) were taken at 0, 30, 60, and 90 days of exposure and samples for selenium tissue residue analysis were taken at 30, 60, and 90 days. Mortality in all treatments were recorded daily. The temperature, pH, hardness, conductivity, oxygen, chloride, and sulfate concentration of the exposure water were determined weekly. The pH, alkalinity, and sulfate concentration were measured on each blended tank of experimental water. One-hundred milliliters of exposure water were collected from mid-water column of each treatment and prepared for selenium analysis at day 0 and every two weeks thereafter. Water samples for selenium speciation were taken from the low, medium, and high concentrations at 0, 45, and 90 days.

Progress

The acute toxicity tests with striped bass were conducted during FY88 (Table 1). These tests were conducted in both ASTM soft water and the generic brackish water. In general, water quality appeared to have little effect on the acute toxicity of selenium to striped bass. The various forms of selenium were slightly more toxic to striped bass than to bluegill (see report 837.13 - Reproductive success of bluegill exposed to dietary and waterborne selenium). The inorganic forms of selenium (selenate and selenite) were much less toxic to striped bass than the organic form (seleno-L-methionine). Selenate was less toxic than selenite and the 6:1 mixture exhibited some synergism. These results were consistent with those obtained with bluegill and confirm the importance of determining selenium

Table 1. Acute toxicities of selenate, selenite, seleno-L-methionine, and a 6:1 mixture of selenate:selenite to striped bass. Values are 96-H LC50's (ug/L Se) with 95% confidence intervals in parentheses.

Selenium form	Water Quality ASTM soft water	 Brackish water
selenate	39000 (32000 - 49000)	ND[1] (26000 - 43000)
selenite	1320 (960 - 1930)	5360 (4350 - 6560)
seleno-L-methionine	ND 2.3 - 6.5	ND 3.9 - 2.3
6:1 selenate:selenite	ND (7800 - 21600)	ND (13000 - 21600)

1. ND - Not Determined. For these tests LC50's could not be calculated. The ranges provided are conservative estimates of the 95% confidence interval.

speciation when attempting to assess its environmental impact.

During the partial chronic toxicity study, mortality and growth of striped bass were not affected by selenium concentrations up to 3.0 mg/L. Fish exposed at 3.0 mg/L had average whole body residues of 6.82 ug/g Se with a corresponding bioconcentration factor of 2. These results indicate that under chronic exposure conditions striped bass are more resistent to waterborne selenium than bluegill which incurred significant mortality at concentrations of less than 1 mg/L selenium. This difference in selenium tolerance is difficult to interpret since striped bass were slightly more sensitive than bluegill in the acute toxicity tests. However, the species specificity of selenium toxicity under different exposure regimes provides support for the use of multiple species tests in the hazard assessment process.

Work Remaining

Residue samples from the acute toxicity test will be analyzed during the next year. In addition, data analysis from both the acute and chronic toxicity studies will continue and manuscript preparation will begin. No additional toxicity tests are planned at this time.

ACUTE AND CHRONIC EFFECTS OF SIMULATED SAN LUIS DRAIN DISCHARGE WATER ON JUVENILE STRIPED BASS

Due to reductions in funding, these studies have not yet been initiated.

ON-SITE TOXICITY OF TILE DRAINWATER TO EARLY LIFE STAGES OF CHINOOK SALMON AND STRIPED BASS

Introduction

Laboratory studies have documented the acute and chronic toxicities of trace elements, both singly and in combination, to juvenile life stages of chinook salmon and striped bass. According to preliminary data from these studies, some trace elements (i.e., boron, molybdenum, and selenium) may be toxic at the concentrations present in agricultural subsurface (tile) drainwater from the Westlands Water District (Westlands). The Westlands is composed of 42,000 acres of intensively irrigated lands on the west side of the San Joaquin Valley; tile drainwater in several sumps from this area was recently found to contain >1,000 ppb selenium.

The main purpose of this study was to verify the "safe" concentrations of selenium, boron, and molybdenum predicted from laboratory studies by testing actual tile drainwater from the Westlands. Specific objectives of this study were (i) to determine if prolonged (up to 28 days) exposure of young life stages of chinook salmon and striped bass to tile drainwater resulted in measurable toxic effects, (ii) to determine if the toxicity of drainwater varied over time, based on a series of short-term (96-hr) exposure tests with a standard organism (larval fathead minnow) and standard testing conditions, and (iii) to determine if the toxicity of drainwater is associated with the concentrations of trace elements (i.e., boron, molybdenum, and selenium), the ratios of major ions (i.e., Ca^{+2}, K^+, Mg^+, Na^+, Cl^-, CO_3^{-2}, and SO_4^{-2}), or the salinity.

The on-site tests were originally expected to use drainwater from the San Luis Drain in the vicinity of Kesterson Reservoir. In July 1985, however, flows of tile drainwater from the Westlands to the Drain were halted. Therefore, in order to conduct this study, we substituted tile drainwater collected from a "worst case" sump in the Westlands. Insofar as this study was an attempt to verify the laboratory-derived estimates of "safe" concentrations of trace elements, the use of drainwater from the "worst case" sump seemed to be appropriate for the tests.

Methods

Three types of tests--each differing mostly by the length of exposure (i.e., 96 hrs, 7 days, and 28 days)--were conducted during this study. The methodology for these tests are briefly described below.

Chinook salmon (40-50 mm total length) and striped bass (30-40 mm total length) fingerlings for the 28-day tests were obtained from hatcheries operated by the California Department of Fish and Game (DFG). Larval striped bass (0-day-old) for the 7-day test and larval fathead minnows (0 to 3-day-old) for the 96-hr tests were obtained from the DFG and NFCR-Columbia, respectively.

The salmon and striped bass were exposed to serial dilutions (0, 12.5, 25, 50, and 100%) of tile drainwater (WWD), reconstituted drainwater (RWWD; contained approximately the same concentrations of major ions as WWD water but lacked elevated concentrations of boron, molybdenum, and selenium), and reconstituted seawater (IO; 100% IO had approximately the same conductivity as 100% WWD and 100% RWWD). All dilutions were made with water from either the San Joaquin River (SJR) for the test with chinook salmon, or the Sacramento-San Joaquin Delta (DELTA) for the tests with larval and fingerling striped bass, or a standard EPA "very hard" water for tests with larval fathead minnows.

WWD water was collected from Sump #20 in the Westlands (Note: based on ranked measurements of boron, molybdenum, selenium, and conductivity taken on 8 occasions in 1986-87, this sump was identified as the "worst" of 63 active sumps). Waters for RWWD and IO were formulated to meet the approximate concentrations of major ions in WWD water and dilute seawater, respectively.

The 28-day tests were conducted as static tests, with daily renewal of the treatments (test solutions). On the other hand, the 7-day test with striped bass larvae and the 96-hr tests with fathead minnow larvae were conducted as static tests without renewal.

For the 28-day tests, new batches of water were collected or reconstituted once every four days. At that time, water samples were preserved for analysis of trace elements. Dead fish in each test chamber were collected and counted daily, then saved for trace element analysis. On Day 0, all fish were measured for total length and weight. On Days 14 and 28, the surviving fish were remeasured in order to document differences in growth rates. On Day 28, the surviving fish also were saved for trace element analysis.

Each time that WWD water was collected, a 96-hr test with fathead minnow larvae was conducted. The specific intent of the 96-hr tests was to identify and document any changes in the toxicity of WWD water during the time periods covered by the 28-day and 7-day tests.

Progress

Preliminary analysis of the data was attempted only with a portion of the results obtained from the 28-day tests. This analysis indicates that, after 28 days, over three-fourths of the salmon exposed to full-strength (100%) WWD water had died, whereas all of the salmon exposed to the other treatments were still alive (Fig. 1). A dose response was observed in the growth of salmon (as measured through comparisons of total length and weight), with the poorest growth occurring at the highest concentrations of both WWD and RWWD waters (Figs. 2-3). A similar dose response was not evident in salmon exposed to IO water.

None of the striped bass exposed to full-strength WWD water survived beyond 23 days, and only about 5% of the fish exposed to full-strength RWWD water were alive after 28 days (Fig. 4). In addition, the survival of fish in both WWD and RWWD (but not IO) waters seemed to be dose-related. Although not significantly different ($\underline{P}$ >0.05) from controls, the growth in weight (but not length) of striped bass seemed to be affected by RWWD water in a dose-related pattern (Figs. 5-6).

Although statistical analysis and interpretation of the data are still in progress, these preliminary observations strongly suggest that tile drainwater is toxic to both chinook salmon and striped bass. Moreover, part of the toxicity might be due to the unusual ionic composition of drainwater. In addition, salinity does not appear to contribute substantially to the toxicity of drainwater. We have not yet evaluated the role that trace elements might play in the toxicity of tile drainwater because water samples are still awaiting chemical analysis.

Work Remaining

Water, fish food, and fish samples from this study are currently being processed for chemical analysis. Also, data on mortality and growth for all toxicity tests (i.e., 28-day, 7-day, and 96-hr) are still undergoing statistical evaluations.

Figure 1. Survival of chinook salmon fingerlings exposed for 28 days to various treatments. Legend: CONTROL, dilution water from the San Joaquin River; WWD, tile drainwater from Sump #20 in the Westlands Water District; RWWD, reconstituted drainwater; and IO, reconstituted seawater. Codes: *, significantly different from controls (P ≤0.05, based on Dunnett's one-tailed t test).

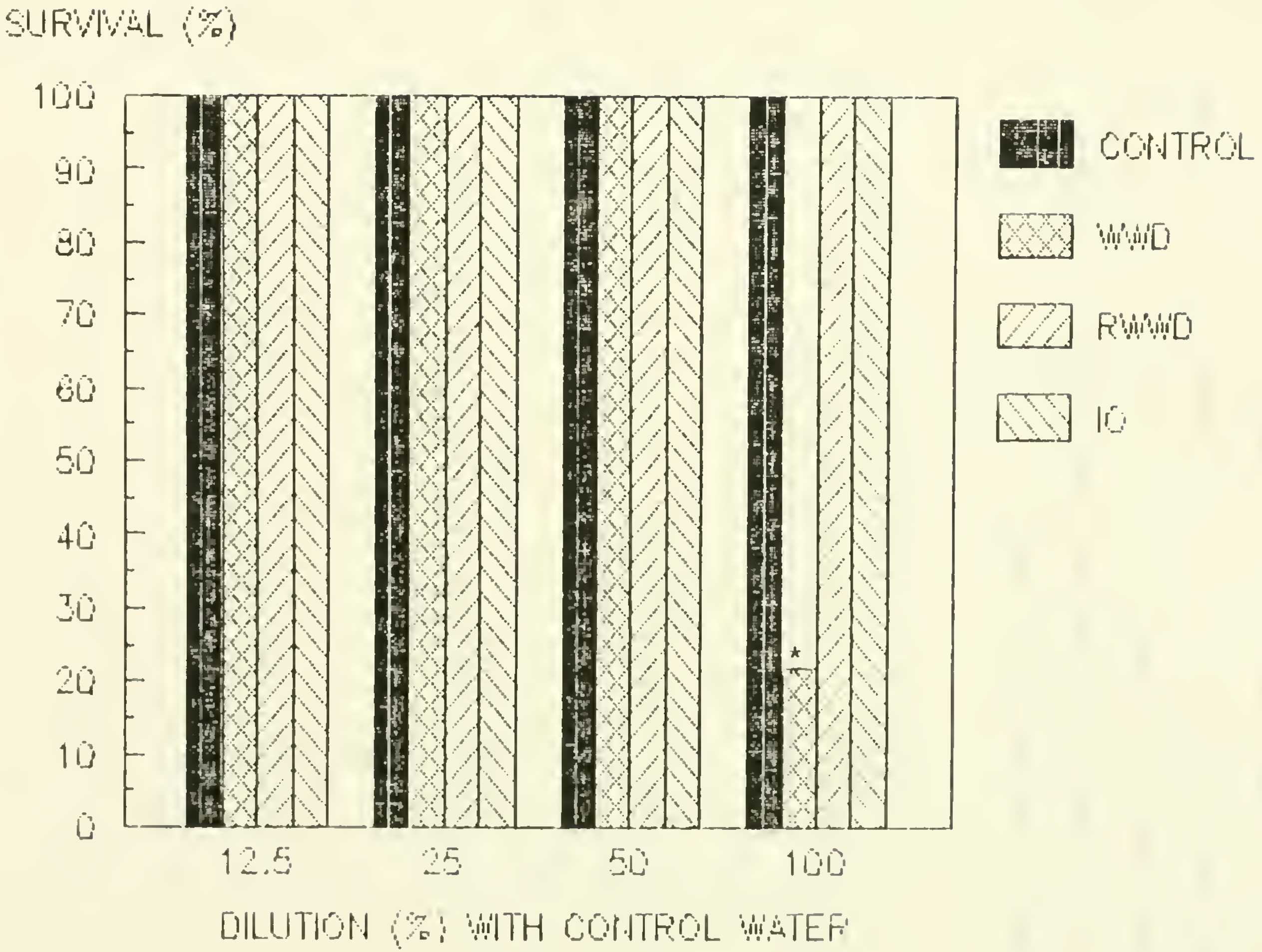

SURVIVAL (%)
100
90
80
70
60
50
40
30
20
10
0
12.5
25
50
100
DILUTION (%) WITH CONTROL WATER
*
CONTROL
WWD
RWWD
IO

Figure 2. Total lengths attained by chinook salmon fingerlings exposed for 28 days to various treatments. Legend: CONTROL, dilution water from the San Joaquin River; WWD, tile drainwater from Sump #20 in the Westlands Water District; RWWD, reconstituted drainwater; and IO, reconstituted seawater. Codes: *, significantly different from controls (*P* ≤0.05, based on Dunnett's two-tailed *t* test).

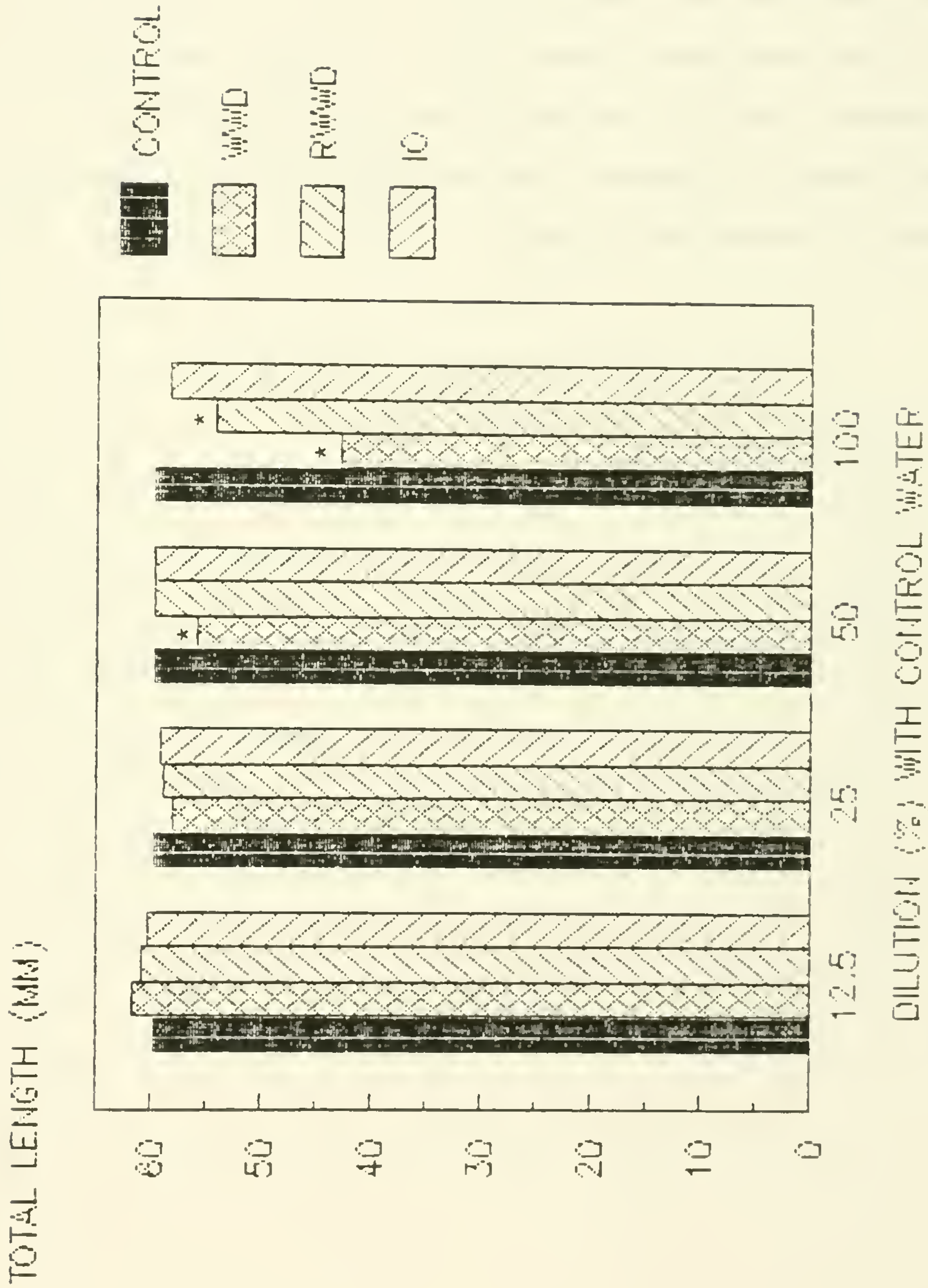

TOTAL LENGTH (MM)
60
50
40
30
20
10
0
12.5
25
50
100
DILUTION (%) WITH CONTROL WATER
*
*
*
CONTROL
WWD
RWWD
IO

Figure 3. Weights attained by chinook salmon fingerlings exposed for 28 days to various treatments. Legend: CONTROL, dilution water from the San Joaquin River; WWD, tile drainwater from Sump #20 in the Westlands Water District; RWWD, reconstituted drainwater; and IO, reconstituted seawater. Codes: *, significantly different from controls (*P* ≤0.05, based on Dunnett's two-tailed *t* test).

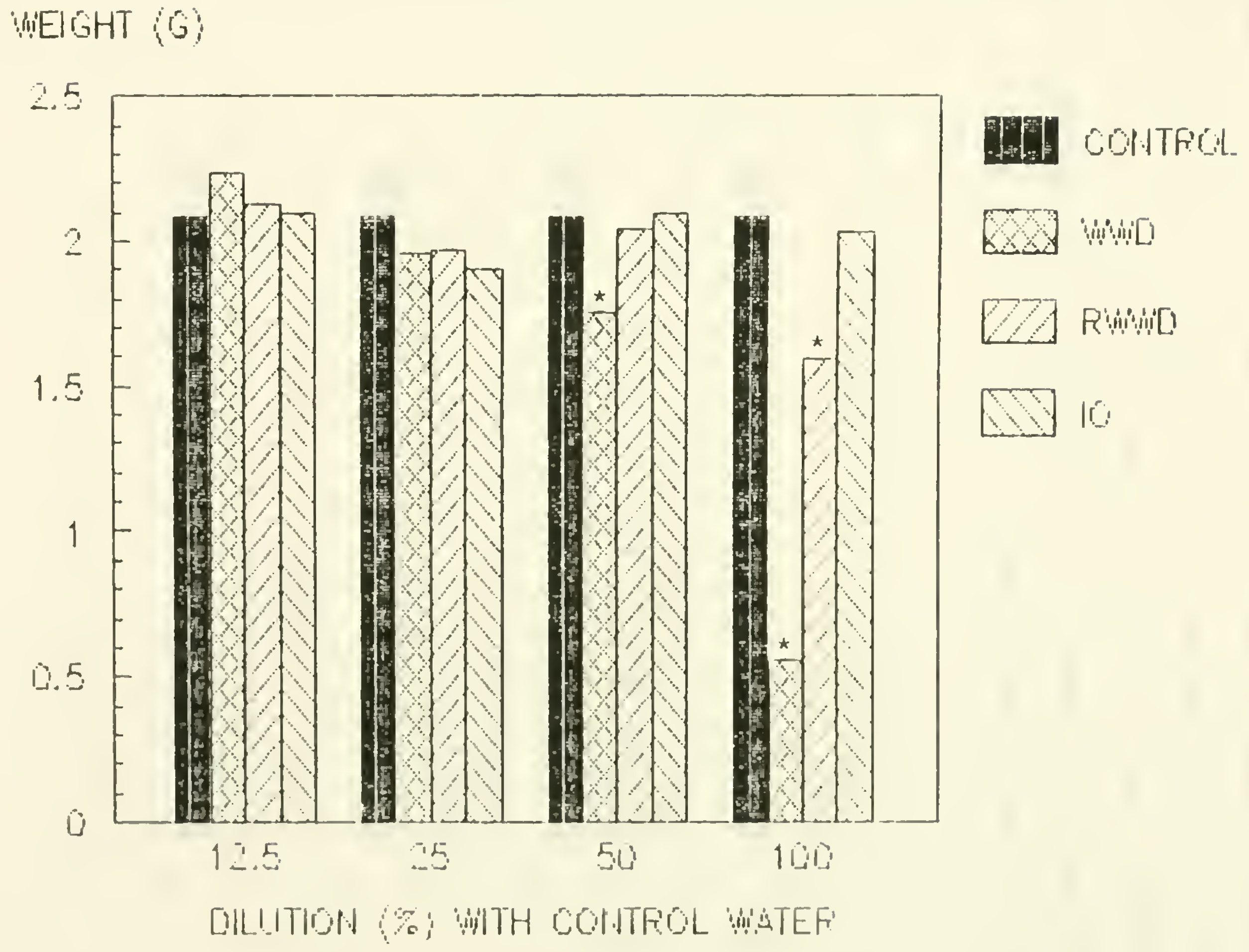

WEIGHT (G)
2.5
2
1.5
1
0.5
0
12.5
25
50
100
DILUTION (%) WITH CONTROL WATER
*
CONTROL
WWD
RWWD
IO

Figure 4. Survival of striped bass fingerlings exposed for 28 days to various treatments. Legend: CONTROL, dilution water from the Sacramento-San Joaquin Delta; WWD, tile drainwater from Sump #20 in the Westlands Water District; RWWD, reconstituted drainwater; and IO, reconstituted seawater. Codes: *, significantly different from controls ($P \leq 0.05$, based on Dunnett's one-tailed t test).

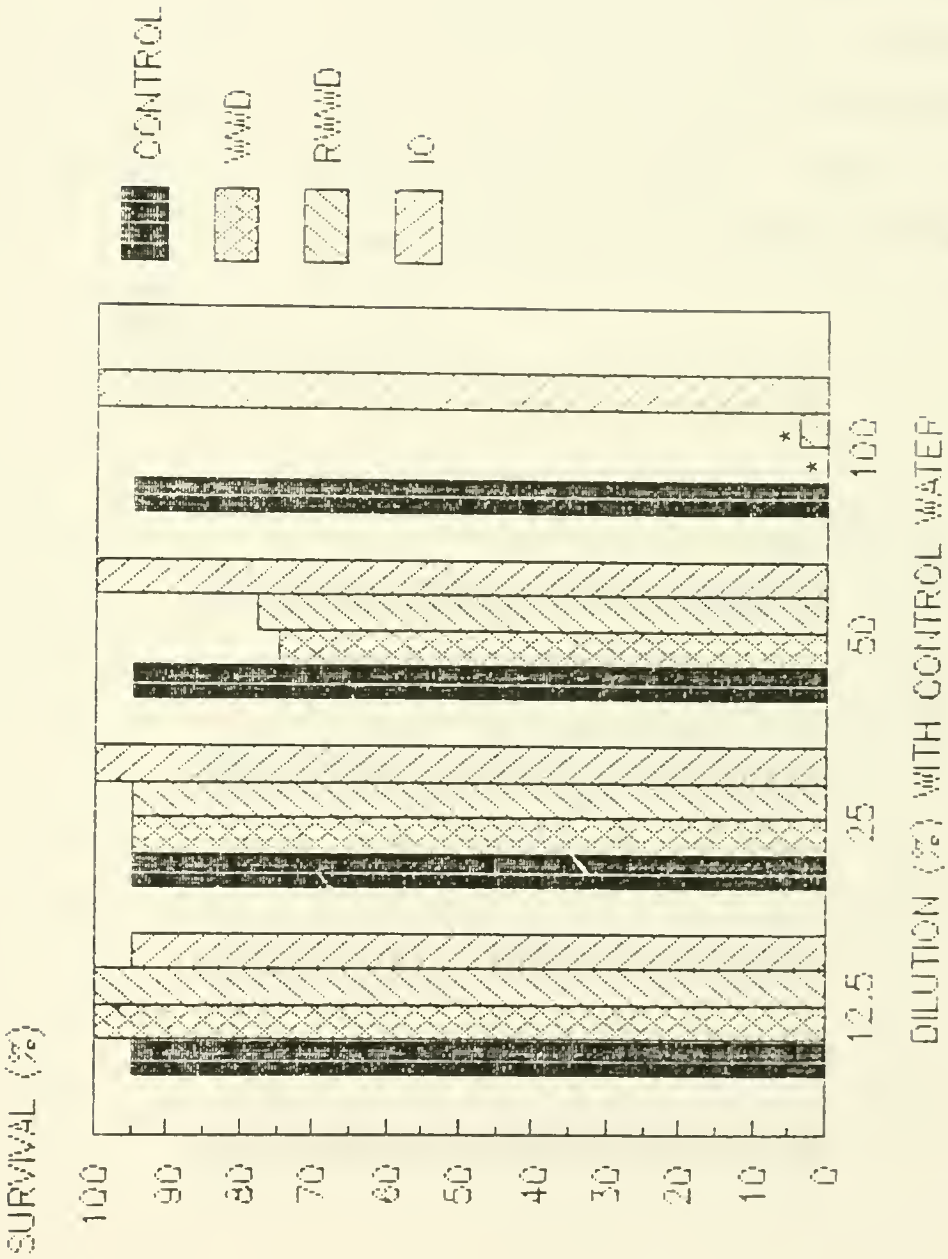

SURVIVAL (%)
100
90
80
70
60
50
40
30
20
10
0
12.5
25
50
100
DILUTION (%) WITH CONTROL WATER
*
*
CONTROL
WWD
RWWD
IO

Figure 5. Total lengths attained by striped bass fingerlings exposed for 28 days to various treatments. Legend: CONTROL, dilution water from the Sacramento-San Joaquin Delta; WWD, tile drainwater from Sump #20 in the Westlands Water District; RWWD, reconstituted drainwater; and IO, reconstituted seawater. Codes: ND, no data; *, significantly different from controls (P ≤0.05, based on Dunnett's two-tailed t test).

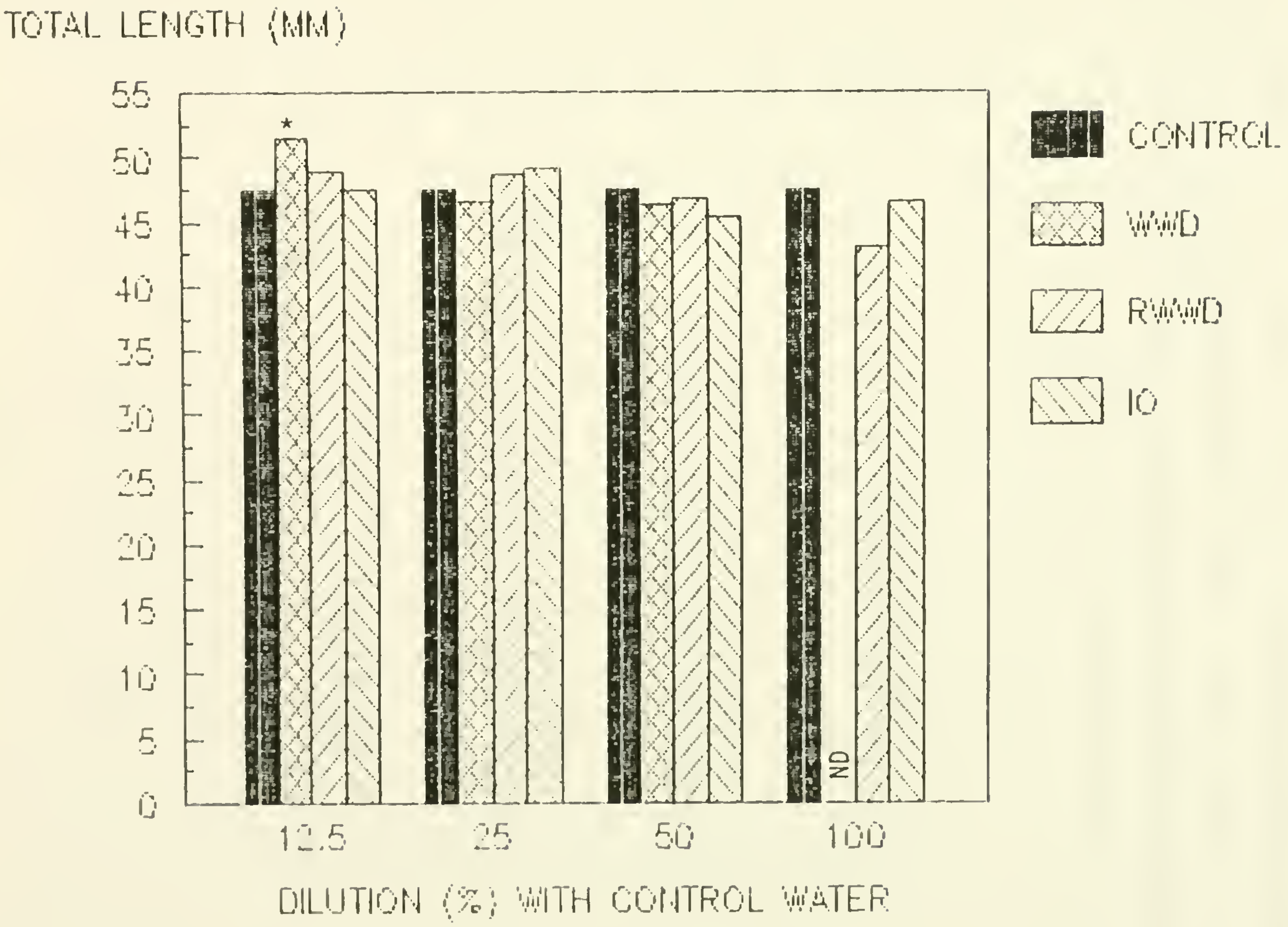
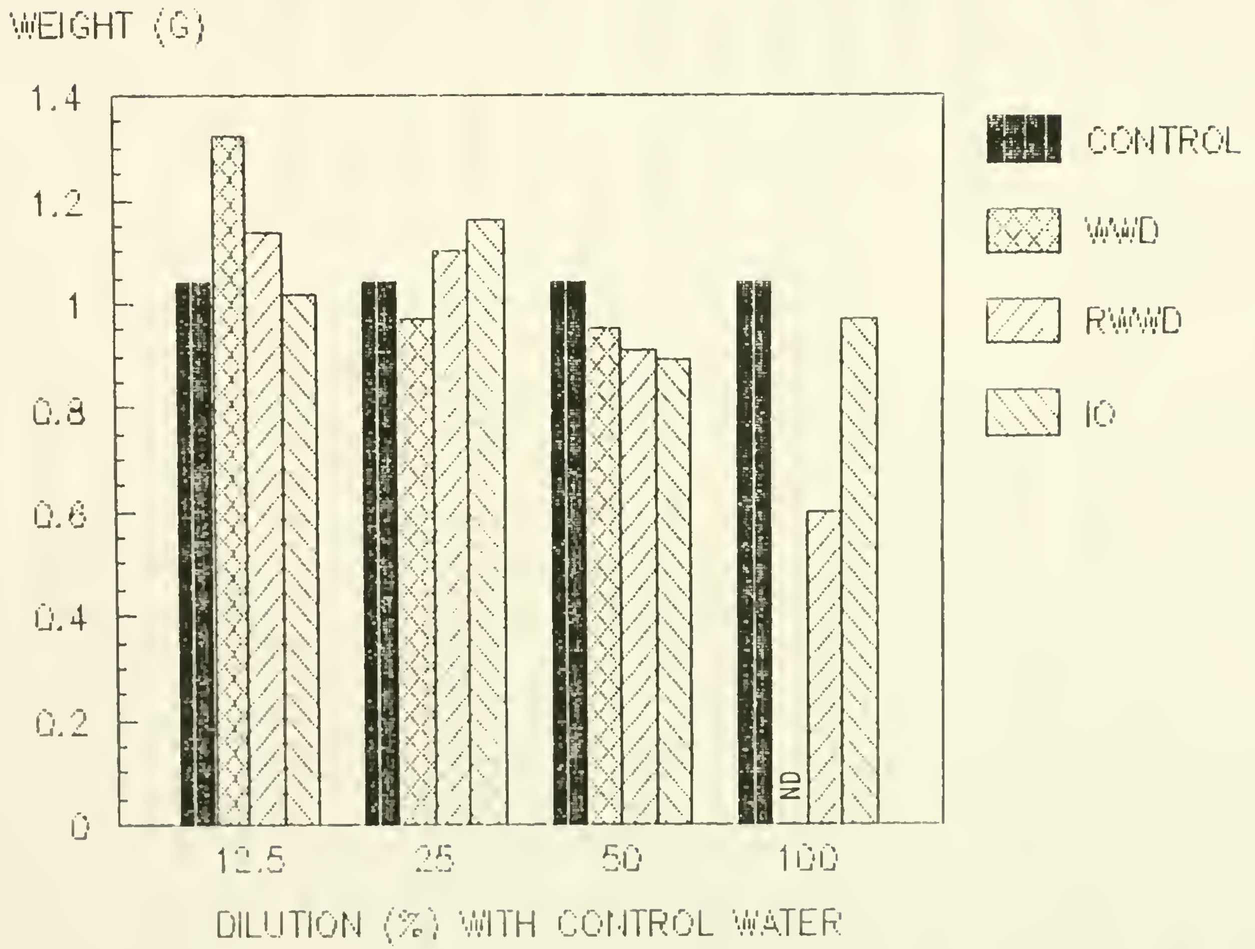

TOTAL LENGTH (MM)
55
50
45
40
35
30
25
20
15
10
5
0
*
ND
12.5
25
50
100
DILUTION (%) WITH CONTROL WATER
CONTROL
WWD
RWWD
IO

Figure 6. Weights attained by striped bass fingerlings exposed for 28 days to various treatments. Legend: CONTROL, dilution water from the Sacramento-San Joaquin Delta; WWD, tile drainwater from Sump #20 in the Westlands Water District; RWWD, reconstituted drainwater; and IO, reconstituted seawater. Codes: ND, no data. Note: none of the weights from the various treatments differed significantly from those of the controls ($P > 0.05$, based on Dunnett's two-tailed t test).


WEIGHT (G)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
ND
12.5
25
50
100
DILUTION (%) WITH CONTROL WATER
CONTROL
WWD
RWWD
IO

EFFECTS OF SELENIUM ON BLUEGILL REPRODUCTION

Introduction

The U.S. Bureau of Reclamation began the Central Valley Project in California in 1935 to provide irrigation service to owners of farms of 160 acres or less. Today, the Shasta Dam located on the upper Sacramento River, in addition to several other dams, reservoirs, canals and conveyances constitue the the Central Valley Project.

Due to the seleniferous nature of the region's soil, selenium can accumulate to potentially toxic concentrations in irrigation water. Selenium enriched irrigation water may pose a threat to fish and wildlife inhabiting the region. Recently, selenium has been measured at levels up to 1350 ug/L in evaporative basins, which greatly exceeds worldwide ambient levels in rivers (0.2 ug/L) and seas (0.09 ug/L).

One specific area of concern is the effect of selenium on the reproductive sucess of two economically important anadromous species; striped bass and chinook salmon. To asses this concern the National Fisheries Contaminant Researh Center, through an intra-agency agreement with the Bureau of Reclamation conducted a series of acute and chronic toxicity tests intended to evaluate the relative toxicity of various forms of selenium to fish. The results of these studies were used to design a reproduction study with bluegill sunfish (_Lepomis_ _macrochirus_) to evaluate the potential effect of selenium on fish reproduction. Bluegill were chosen for use in the reproductive study as serogates for the species of concern for a variety of reasons. Bluegill are widely distributed in the Valley, they have been documented as being reproductivly sensitive to selenium and their relatively simple reprocuctive cycle is condusive to labororatry manipulation. This document provides a review the methods and results of the acute and chronic toxicity tests completed in FY 87. It also reviews the methods used in the reproductive study began in FY 87 and completed in FY 88 and summarizes results of the bluegill reproduction study.

Methods

I - Acute toxicity studies: A series of acute toxicity studies with bluegill were conducted during FY 87 in both a generic freshwater (described later) and standard ASTM soft water (hardness, 40-48 mg/L as CaCO3; alkalinity, 30-35 mg/L as CaCO3; pH, 7.2-7.6). Under static conditions bluegill were exposed for 96-hours to water-borne sodium selenate, sodium selenite, a 6:1 mixture of sodium selenate to sodium selenite, seleno-L-methionine, and seleno-DL-methionine. The studies were conducted in 19.6 L glass jars containing 15 L of exposure water. Treatments were spiked to obtain the desired exposure concentrations and there was a 60% dilution factor between each concentration. Ten fish were placed into each jar and mortality was monitored every 24 hours. Water samples were taken from each exposure at 0 and 96-hours of the studies and analyzed for total selenium. In addition, filtered samples were taken from the control, low, medium, and high concentrations of the mixture studies and analyzed for selenate and selenite.

II- Chronic toxicity studies: Prior to initiating the bluegill reproductive study, three range-finding partial chronic toxicity studies with selenium were conducted in flow-through proportional diluters. These studies included: 1) a 30 day water-borne selenium study, 2) a 60 day water-borne selenium study, and 3) a 90 day selenium diet study. All studies were conducted using a generic fresh-water whose inorganic composition mimicked waters existing in the San Joaquin Valley. The water was prepared by blending appropriate amounts of well, reverse osmosis (RO) water, calcium sulfate, and magnesium sulfate in 8327 L polyolefin tanks to yield a final experimental water containing 28 mg/L calcium, 14 mg/L potassium, 5 mg/L chlorides, 72 mg/L sulfates, 60-65 mg/L alkalinity as $CaCO_3$, 134 mg/L hardness as $CaCO_3$, and a pH of about 8.1. Before initiating the studies, fish were acclimated from well water to experimental water quality over a 5-day period.

A. Water-borne selenium study (30 day): In the first chronic toxicity study, young bluegill about 3 months old were exposed to a 6:1 water-borne mixture of selenate to selenite for 30 days. The study was conducted at 25°C and the fish were fed a commercial diet 3 times daily. Sodium selenate (98% pure, 41.8% selenium) and sodium selenite (98% pure, 45% selenium) stock solutions were prepared in RO water at concentrations corrected for % purity and % selenium in the compounds. Nominal exposure concentrations of selenium in the 6:1 mixture were 18.6, 9.3, 4.5, 2.3, and 1.2 mg/L for selenate and 3.1, 1.6, 0.8, 0.4, and 0.2 mg/L for selenite, constituting total selenium exposure concentrations of 21.7, 10.9, 5.4, 2.7, and 1.4 mg/L. Fish were also exposed to duplicate controls with no selenium added. These exposure concentrations were derived from acute toxicity data for bluegill exposed to the 6:1 mixture and acute and chronic toxicity data on rainbow trout exposed to sodium selenite.

Ten bluegill were placed into each small chamber of the exposure aquaria for growth determinations and 40 fish were placed in each of the larger chambers for tissue residue sampling. Growth samples were taken at 0, 15, and 30 days of exposure, and samples for tissue analysis for selenium were taken at 30 days. Mortality in all treatments was recorded daily. The temperature was monitored daily and pH, hardness, alkalinity, conductivity, chlorides, and sulfates of the exposure water were determined weekly. In addition, the pH, alkalinity, hardness, and sulfate were measured on each blended tank of experimental water. Two-hundred mL of exposure water were collected from mid-water column of each treatment and prepared for selenium analysis at day 0 and every two weeks thereafter. Four-hundred mL of exposure water from the low, medium, and high concentrations were prepared for selenium speciation analysis on day 0 and 30.

B. Water-borne selenium study (60 day): In the second chronic toxicity study, young-of-the-year bluegill were exposed to water-borne selenium for 60 days. The exposure procedures for the second study were identical to those of the first, except that the selenium exposure concentrations were lower, the study lasted 60 days, and the bluegill were approximately 5 months old. Nominal exposure concentrations of selenium in the 6:1 mixture were 0.00, 0.146, 0.293, 0.585, 1.17, and 2.34 mg/L for selenate, and 0.00, 0.024, 0.049, 0.098, 0.195, and 0.39 mg/L for selenite, constituting total selenium exposure concentrations of 0.00, 0.171, 0.341, 0.683, 1.38, and 2.73 mg/L. Mortality was monitored daily and samples for growth (length and weight) were taken at 0, 15, 30, and 60 days. Samples for selenium tissue residues were taken at 30 and 60 days. At 60 days, surviving fish were placed in selenium-free water and sampled at 3, 7, 14, and 28 days to determine selenium depuration rates.

C. Selenium diet study (90 day): In the third chronic toxicity study, 3 month old bluegill were exposed to a selenium permeated diet for 90 days. The bluegill were fed seleno-L-methionine permeated into a salmon-meal-base diet and a control treatment of the same diet with no seleno-L-methionine added. Nominal exposure concentrations of seleno-L-methionine in the test diet were 26.0, 13.0, 6.5, 3.3, and 1.6 mg/kg. These concentrations were selected on the basis of results from an earlier study with chinook salmon exposed to a seleno-L-methionine permeated diet. Prior to initiation of the feeding study, the bluegill were acclimated to the control diet over a one week period, and then to experimental water and temperature

(25°C) over a 4-day period. Based on 0-day growth samples, the bluegill were fed the test diet at a rate of 3% of the biomass per test chamber per day in three equal feedings. Feeding rate was adjusted to the control treatment biomass after each growth sampling period. Sampling procedures in this study were the same as those described above.

III- Bluegill Reproduction Study: Two year-old pond cultured bluegill were treated prophylactically with 6 g/L NaCl for one week. During treatment and acclimation, fish were held in well water (pH, 7.8; hardness, 283 mg/L as $CaCO_3$; alkalinity, 255 mg/L as $CaCO_3$) at 22°C. The study was initiated by distributing 28 adult bluegill into each of 12 stainless steel (630 liter) exposure tanks of a modified proportional diluter. During the first 24 hours of the study, fish were exposed to 5 duplicated treatments containing 10 µg/l total selenium (a 6:1 mixture of selenite and selenite) established in laboratory well water. Fish were also exposed to duplicated control conditions containing no waterborne selenium. Total waterborne selenium concentrations of the 6:1 mixture contained 8.6 and 1.4 ug/L selenate selenite respectively. After the initial 24 hour period, a generic fresh-water that was representative of waters in the San Joaquin Valley was substituted for the well water and used as diluent for the duration of the study.

Bluegill in two treatments were fed control diet (containing no seleno-L-methionine). Fish in one of the two treatments recieveing control diet were exposed to 10 ug/L waterborne selenium. Fish in the four remaining treatments were exposed to 10 ug/L of waterborne selenium were fed one of four seleno-L-methionine diets. Mean measured seleno-L-methionine concentrations in the diets were 33.3. 16.8, 8.45 4.63 µg/g (dry weight). The adult bluegill were exposed to this treatment regime for a total of 140 days.

The first 60 days of the 140 day adult exposure comprised the pre-spawning phase of the study. During this period, lighting and temperature was controlled so as not to elicit spawning in the adult fish. A 12:12 light to dark photoperiod was maintained and the temperature was held at 22°C. Near the end of the pre-spawning phase, the photoperiod was adjusted to 16 hours light and 8 hours dark and the temperature was gradually increased to 28°C to achieve optimum conditions for bluegill spawning. During the second 90 days of the study (spawning phase), photoperiod and temperature was maintained to sustain the optimum spawning conditions for the fish.

During the spawning phase, the effects of water-borne and dietary selenium on the reproductive success of bluegill was determined by measuring the frequency of spawning, number of eggs per spawn (fecundity), hatching success of selected eggs, and survival of resultant fry for 30 days. Fecundity was determined for each spawn by removing the eggs from the substrate, measuring the total volume of eggs in the spawn and then counting a 10 percent volume of the eggs using a Biotran_R colony totalizer. The number of eggs per volume of subsample were multiplied by the total volume of eggs in the spawn to estimate the total number of eggs per spawn.

The hatching success of eggs from each spawn was determined throughout the spawning phase of the study. After careful removal of from the substrate, the eggs were transferred to a glass dish and examined with a dissecting scope for evidence of embryonic differentiation. Once selected, twenty viable eggs were transferred from the glass dish using an eye dropper into water contained in each of four hatching cups. Twenty viable eggs were transferred into each hatching cup. The cups were suspended in the adult exposure system in the treatment where the spawn occurred until the eggs hatched (approximately 16 hours post deposition). After hatching, the number of living and dead fry was recorded.

To determine fry survival for selected spawns, five fry were removed from each hatching cup (20 fry per spawn) and transferred to a growth chamber in a second diluter with waterborne selenium concentration corresponding to the parental exposure. Evaluation of fry survival was limited to four sequential spawns at one time from each treatment group of adult bluegills during a 30 day period.

All fry in the growth chambers of the second diluter were automatically fed brine shrimp ad libitum every 30 minutes. Starting at day 15 post-hatch, the fry were fed the seleno-L-methionine diet corresponding to their parental diet treatment in addition to brine shrimp. Daily observations of mortality and were recorded. The length of fry was determined photographically on day 15 post-hatch. At 30 days post-hatch, surviving fish were weighed, measured for total length, and frozen for whole-body selenium residue analyses.

Progress

I-Acute toxicity studies: The acute toxicity studies with bluegill exposed to selenate, selenite, seleno-L-methionine, and seleno-DL-methionine, in both freshwater and ASTM soft water as well as the acute toxicity range-finding studies with bluegill exposed a 6:1 mixture of selenate to selenite were completed in FY 87. The acute toxicity of selenium in freshwater and ASTM soft water were similar. While the exposures of bluegill to selenium in the two different water qualities were not designed to determine the effect of sulfate, hardness, and alkalinity on the toxicity of selenium, these parameters did not appear to influence selenium toxiicy. Measured exposure concentrations of selenium at the initiation of each study were approximately 95% of nominal values. Selenium analyses at the end of the studies indicate that the exposure concentrations did not decline substantially during the 96 hours except in the case of the selenomethionine studies where nominal values decreased by 65-75 percent.

The 96-hour LC50's show that selenate is less toxic to bluegill than selenite which is less toxic than both seleno-L-methionine and seleno-DL-methionine. Whereas the two organic forms of selenium showed similar acute toxicities they were both more toxic to bluegill than the inorganic forms. The increased toxicity of the organic forms of selenium compared to the inorganic forms may be related to differences in bioavailability or potential in vitro or in vivo transformation products; however, additional research is required to address this question. In the range-finding studies, the acute toxicity to bluegill of the 6:1 mixture of selenate to selenite was intermediate between that of the inorganic forms singly and the organic forms of selenium. These results imply that synergistic interactions of the two inorganic forms may occur in bluegill. Thus, additional research on the interactive toxicity of various selenium species would also be desirable. The results of the acute studies are summarized in table 1.

Table 1. Acute toxicity as 96-hour LC50's and 95% confidence interval (mg/L selenium) of various forms of selenium to bluegill. Studies were conducted in both freshwater and ASTM soft water.

	Type of Exposure Water	
Selenium Form	Freshwater	ASTM
Selenate	72-120a	98(80-119)
Selenite	7.8-13.0[a]	7.8-13.0[a]
Seleno-L-methionine	0.009 (0.007-0.011)	0.013(0.0110.016)
Seleno-DL-methionine	0.010 (0.008-0.012)	0.013(0.0110.016)

[a] Estimated range within which 96-hour LC50 occurs.

II- Chronic Studies: Exposure of bluegill to water-borne selenium in the first 30 day toxicity study resulted in significant mortality in the lowest concentration tested (1.4 mg/L). Fish exposed to selenium concentrations of 5.4 mg/L exibited 100% mortality between 15 and 30 days and those exposed to concentrations greater than or equal to 10.9 mg/L exibited 100% mortality between 0 and 15 days. Bluegill that survived for 30 days at concentrations less than or equal to 2.7 mg/L selenium did not show significantly reduced growth.

A chronic no-effect concentration between 0.34 and 0.68 mg/L of waterborne selenium was determined from bluegill mortality in the second 60 day chronic toxicity study (Table 2). During the second study significant effects on the growth of bluegill were not observed, however, there was a trend toward increased growth in all the selenium treatments after 45 days (Table 3).

Table 2. Mean % survival of bluegill exposed to water-borne. inorganic selenium.

Days of	Selenium Exposure Concentration (ug/L)					
exposure	Control	170	340	680	1380	2730
15	100.0	100.0	92.5	85.0*	75.0*	52.5*
30	100.0	90.0	80.0*	60.0*	45.0*	12.5*
45	90.0	87.5	77.5	60.0*	30.0*	2.5*
60	90.0	87.5	77.5	47.5*	30.0*	2.5*

*Significantly different from control treatment ($p \leq 0.05$).

Table 3. Mean length (mm), weight (g), and K factor of bluegill exposed to waterborne inorganic selenium.

Days of exposure and variable	Selenium Exposure Concentration (ug/L)					
	Control	170	340	680	1380	2730
Day 15						
Ln	33.2	32.2	33.4	32.1	32.4	32.8
Wt	0.49	0.44	0.52	0.43	0.44	0.47
K factor	1.3	1.3	1.4	1.3	1.3	1.3
Day 30						
Ln	35.7	35.4	37.8	35.8	35.2	36.8
Wt	0.68	0.65	0.82	0.72	0.64	0.67
K factor	1.4	1.4	1.5	1.4	1.3	1.2*
Day 45						
Ln	37.8	38.3	40.3	38.9	40.3	-
Wt	0.84	0.89	1.08	1.06	1.11	-
K factor	1.5	1.5	1.6	1.7*	1.6*	-
Day 60						
Ln	40.4	40.4	42.7	42.0	43.9	-
Wt	1.04	1.08	1.29	1.18	1.40	-
K factor	1.5	1.5	1.6	1.5	1.6	-

*Significantly different from control treatment ($p \leq 0.05$).

There were no significant dose-related effects on survival of bluegill exposed to seleno-L-methionine concentrations in diet as high as 26 mg/kg (Table 4). However, significant reductions in the K factor, an expression of overall condition, occured among bluegill exposed to 13 and 26 mg/kg selenium in the diet (Table 5).

Table 4. Mean % survival of bluegill exposed to dietary selenomethionine.

Days of exposure	Selenium exposure concentration (mg/kg)					
	Control	1.6	3.3	6.5	13.0	26.0
30	97.5	95.0	95.0	90.0	87.5	95.0
60	97.5	95.0	92.5	77.5*	87.5	82.5*
90	95.0	92.5	90.0	77.5*	85.0	82.5

*Significantly different from control treatment ($p \leq 0.05$).

Table 5. Mean length (mm), weight (g), and K factor of bluegill exposed to dietary selenomethionine.

Days of exposure and variable	Selenium exposure concentration (mg/kg)					
	Control	1.6	3.3	6.5	13.0	26.0
Day 30						
Ln	30.4	30.7	30.5	30.4	29.4	29.4
Wt	0.43	0.45	0.42	0.44	0.39	0.36
K factor	1.5	1.4	1.4	1.4	1.4	1.3*
Day 60						
Ln	34.3	33.6	34.9	33.9	33.2	34.3
Wt	0.63	0.60	0.69	0.62	0.57	0.65
K factor	1.5	1.4	1.5	1.4	1.4	1.4
Day 90						
Ln	40.4	38.7	40.8	38.5	38.5	39.1
Wt	0.95	0.87	0.97	0.84	0.81	0.91
K factor	1.3	1.3	1.3	1.3	1.2*	1.2*

*Significantly different from control treatment ($p \leq 0.05$).

III- Reproduction Study: During FY 88, the active testing phase of the 140 day adult bluegill reproduction study was completed. Approximately 550 fish tissue samples (including, whole body, carcass, gonadal, egg and fry) were collected and submitted to the NFCRC Chemistry Section for selenium analyses.

In addition approximately 140 water samples for total selenium and 55 samples for selenium speciation were also collected and submitted. To date approximately 90 percent of the samples have been completed by NFCRC Chemistry Section.

During the spawning period of the study, a total of 135 spawns occurred in all treatments. Fecundity and hatchability were determined on all but 20 of these spawns. Fry survival to 30 days and growth were evaluated in a total of 73 spawns.

Data on the following measured parameters have been analyzed for adult fish: weight, length, gonadal weights (days 60 and 140), gonadal somatic index (GSI), hematocrit, spawning frequency, and fecundity. The following data for fry have been analyzed: hatchability, survival, length (day 15), length and weight (day 30).

A. Residue analyses: Selenium accumulation in whole fish over the first 56 days of the study is presented in Figure 1. This graph illustrates the positive correlation between increasing selenium tissue concentration and increasing selenium exposure concentration. Figures 2, 3, and 4 present selenium concentrations in carcass and gonadal tissue at day 60 (end of the pre-spawning period) and day 140 (end of the study). These data indicate that selenium concentrations in the surveyed tissues attained equilibrium prior to initiation of the spawning period of exposure. Ovaries accumulated more selenium than either carcass or testes. Selenium concentrations in eggs collected from the spawning substrates reflected ovarian selenium concentrations indicating that seleium in the eggs is deposited during oogenisis rather than post-deposition uptake from the water column (Figure 5).

B. Biological effects: Exposure of 140 days to combinations of dietary and water-borne selenium up to and including 33.3 µg/g selenomethionine dry weight and 10 µg/l total selenium in water did not adversely effect the weight, length, gonadal weight, GSI, hematocrit, spawning frequency or fecundity of adult bluegill. The percent hatch of eggs spawned by the exposed fish was also unaffected, as was the growth of surviving fry.

However, one important biological effect was correlated with adult selenium exposure. Figure 6 presents the mean survival to day 30 of fry from all spawns within exposure treatments. Despite the fact that considerable variation in fry survival within and between exposure groups was common, a consistent failure of fry to survive was evident in all spawns resulting from parents exposed to the highest combination of water-borne and dietary selenium. Unlike their counterparts in other exposure groups, these fish exhibited a high rate of mortality prior to the critical period in their development when they switched from internal (yolk sac) feeding to external food sources.

These results suggest that exposure of fish to selenium combinations in the water and diet that may not result in overt toxicological responses in adults may result in reproductive failure by severely reducing the survival of their offspring. Such a striking reproductive effect may help explain the mechanism by which the fisheries are lost in selenium contaminated waters.

This study has contributed to a better understanding of the effect of selenium on fish reproduction. In addition to documenting an apparent reproductive effect, the results of tissue residue analyses may provide a tool by which feral bluegill populations can be evaluated for selenium related reproductive effects.

Work Remaining

Statistical analyses and interpretation of selenium residue data remains to be completed. This analyses is particularly important as it may provide a gonadal tissue selenium concentration for use as comparison benchmark for monitoring, predicting, or diagnosing reproductive impacts in selenium exposed fish populations. Also, interpretation of the tissue residue data in conjunction with the results of other selenium related research (including food chain transfer studies) will contribute to an estimation of "not to exceed" food chain and fish tissue selenium concentrations.

Manuscripts pertaining to the partial chronic waterborne and dietary selenium exposures and the reproductive study are in preparation.

Fig. 1. Mean Whole Body Residues of Bluegill
Exposed to Combined Water-Borne & Dietary Selenium

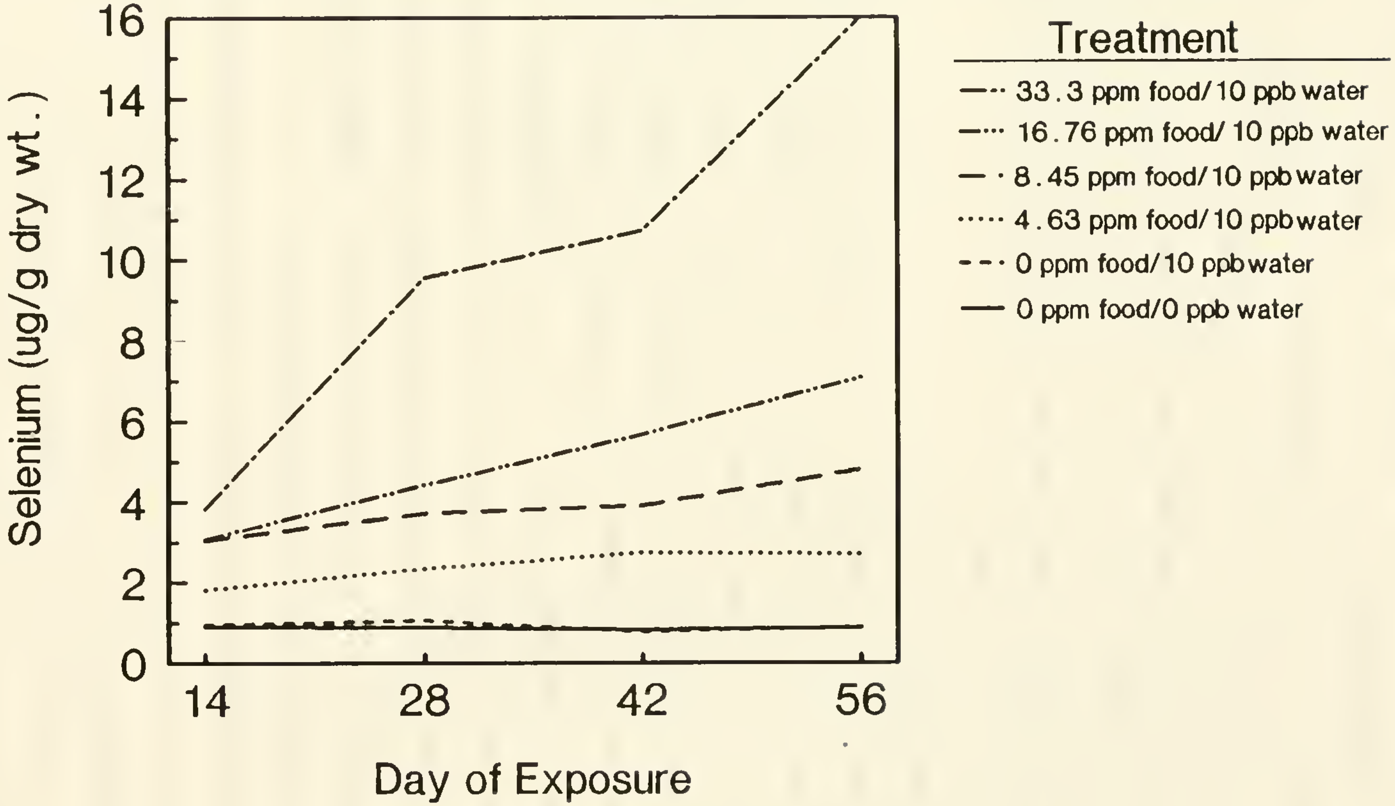


Fig. 2. Mean Carcass Residues of Bluegill Exposed to Combined Water-Borne & Dietary Selenium

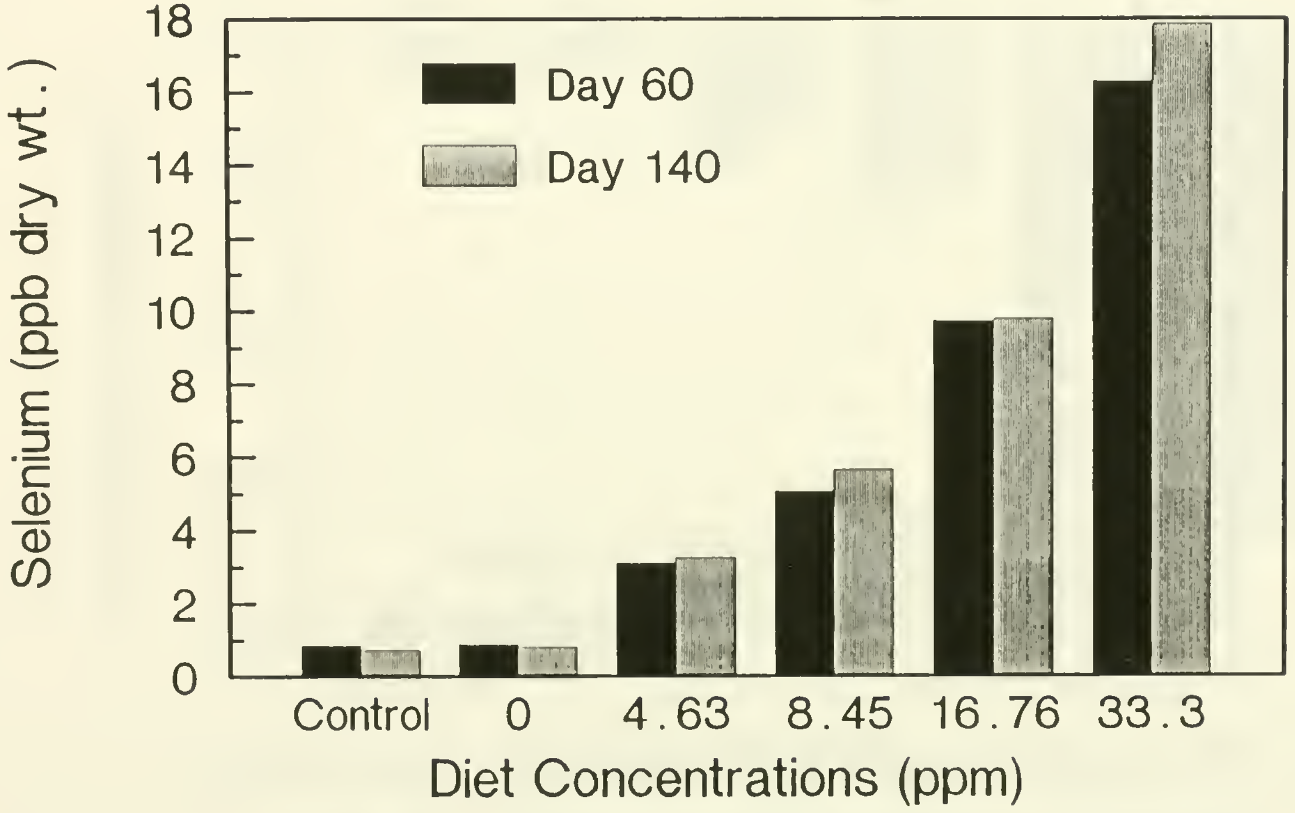


Fig. 3. Mean Ovary Residues of Bluegill Exposed to Combined Water-Borne & Dietary Selenium

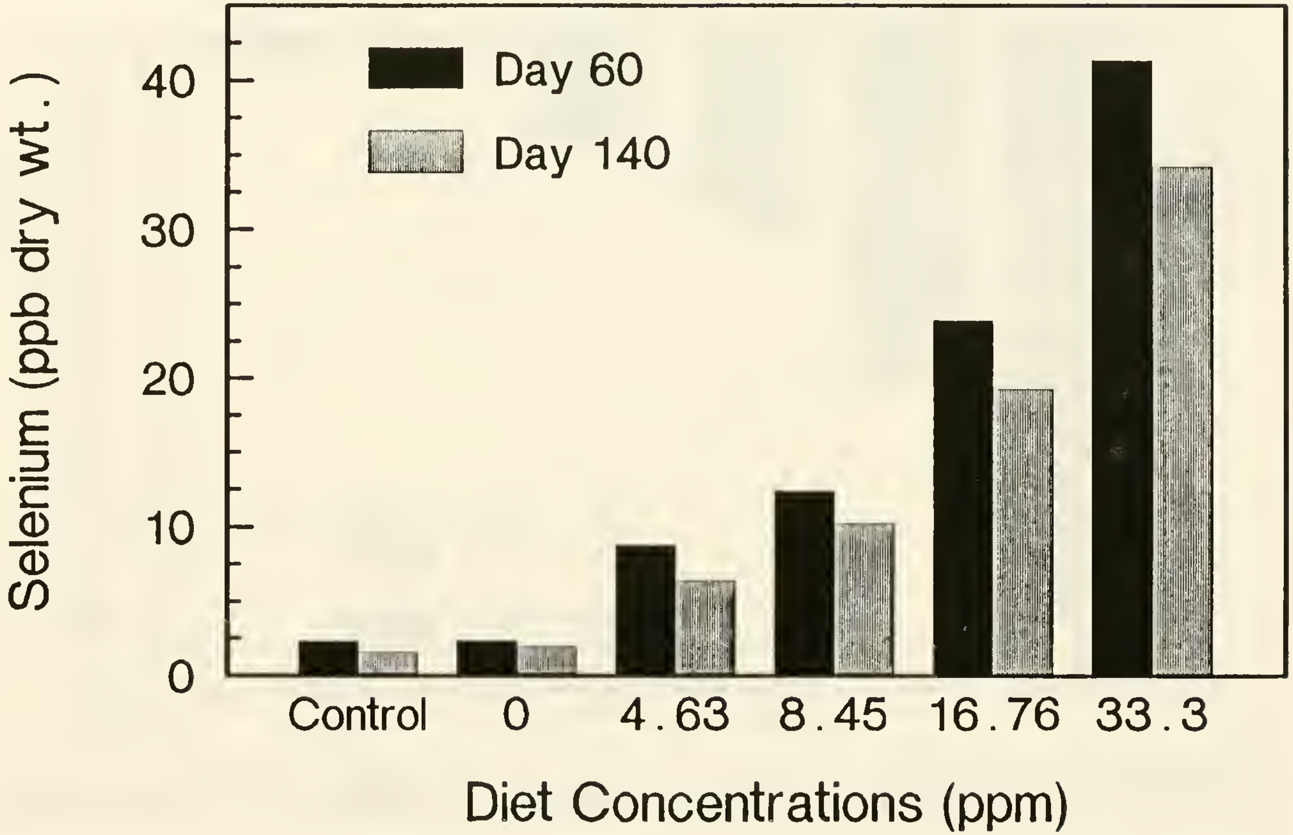


Fig. 4. Mean Testes Residues of Bluegill
Exposed to Combined Water-Borne & Dietary Selenium

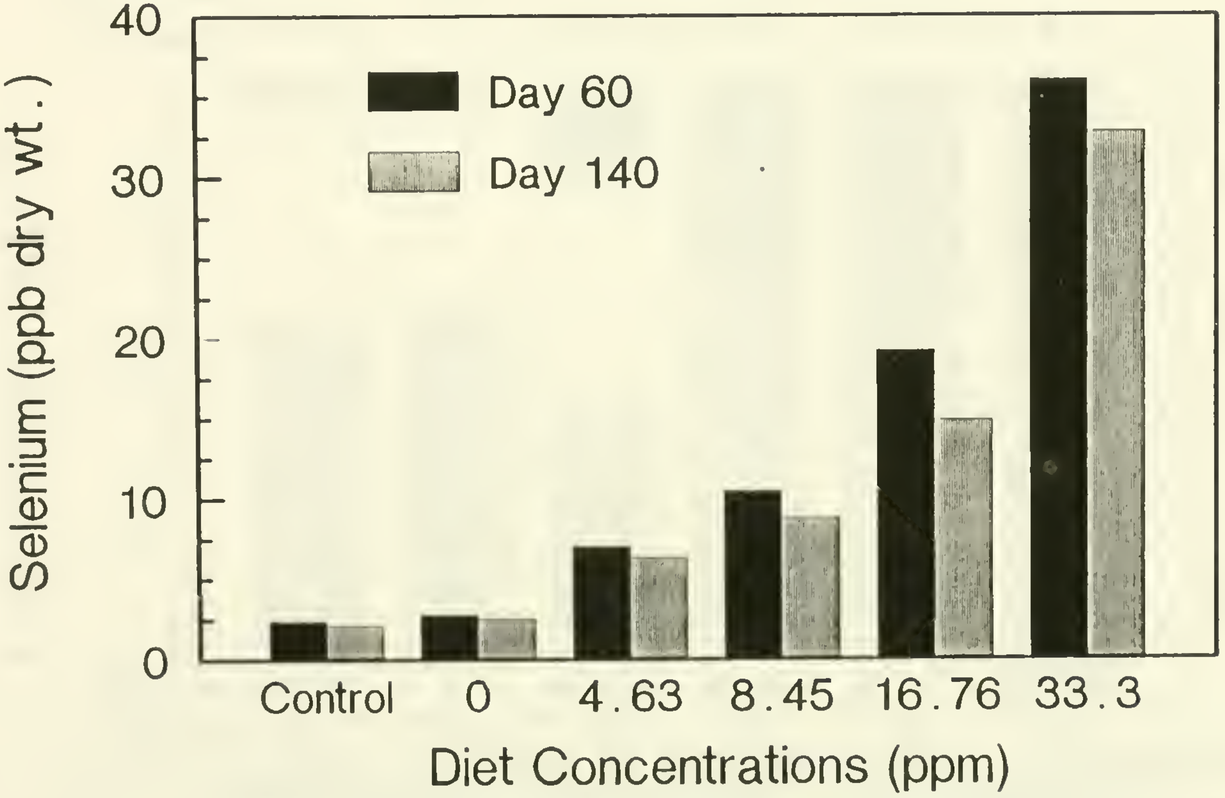


Fig. 5. Mean Egg Residues of Bluegill Exposed to Combined Dietary & Water-Borne Selenium

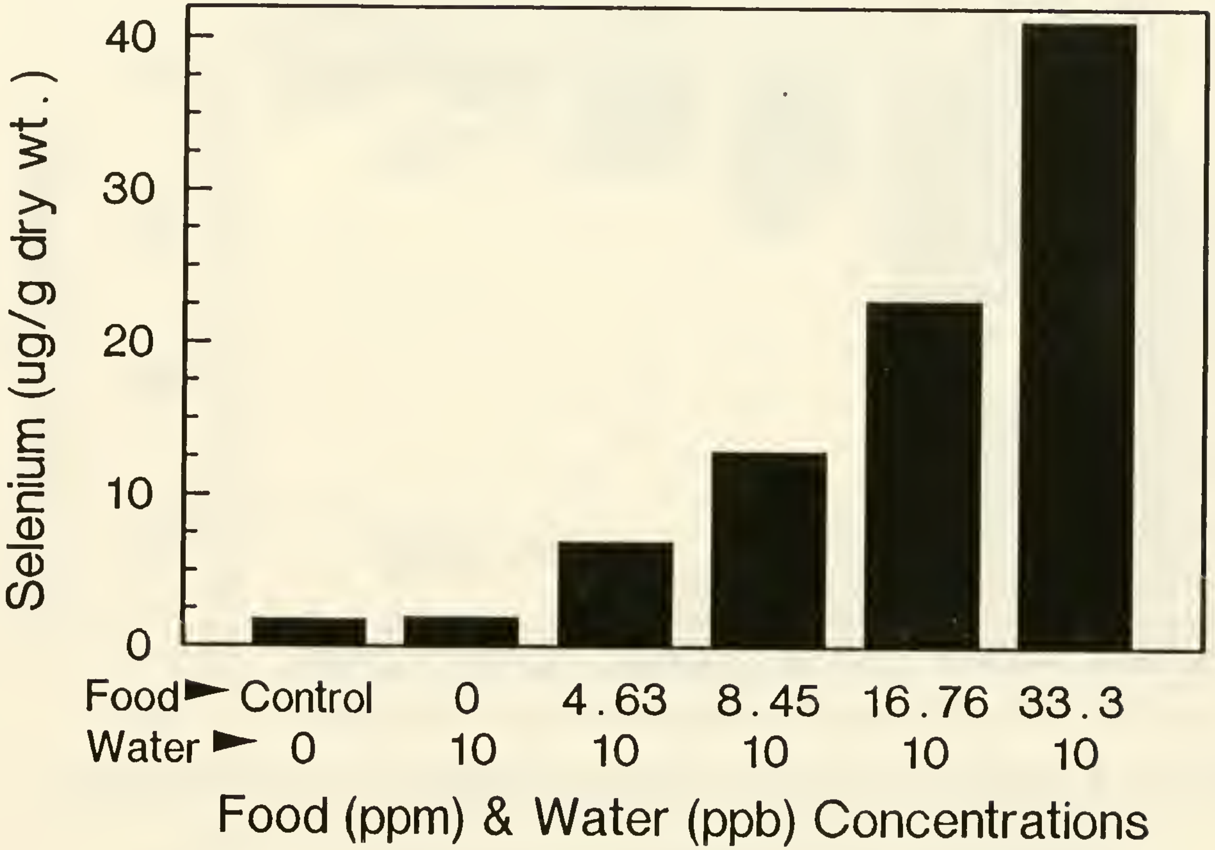
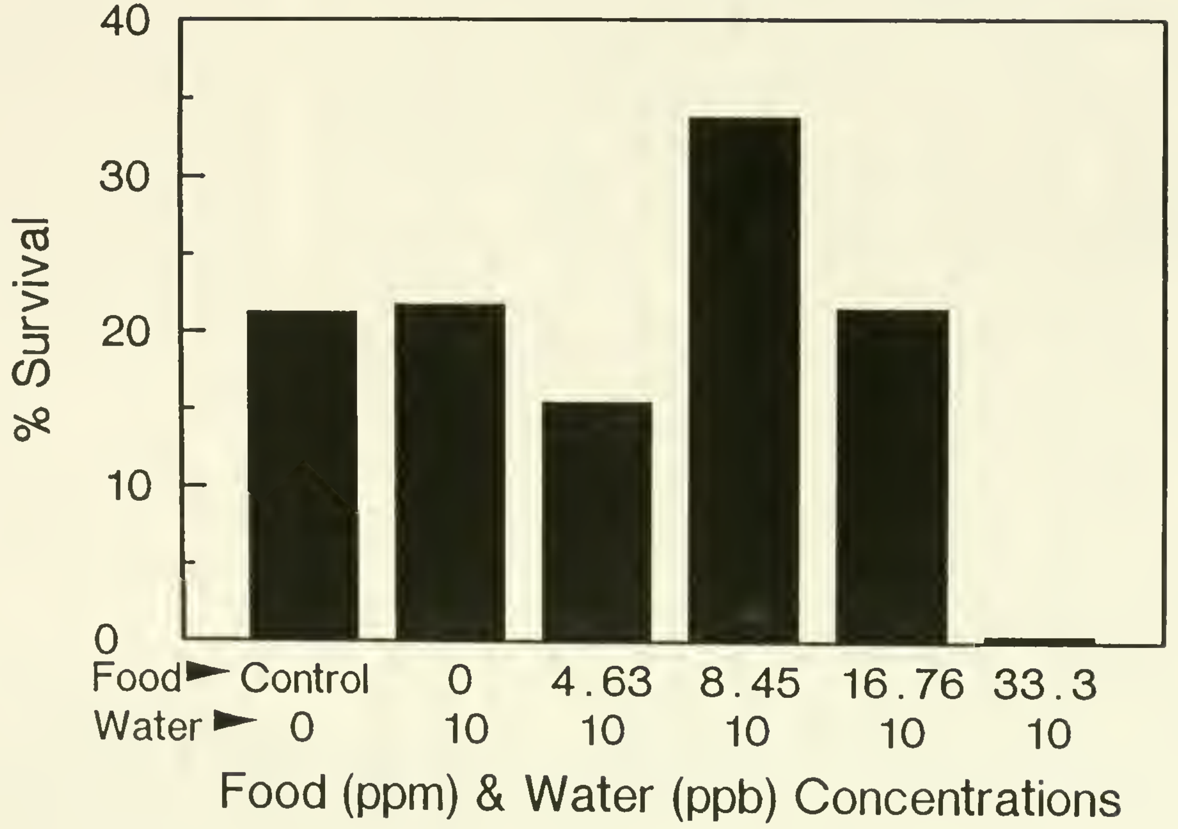


Fig. 6. Mean Survival at Day 30 of Fry from Adults Exposed to Dietary & Water-Borne Selenium



LABORATORY AND MICROCOSM STUDIES OF SELENIUM FATE AND BIOACCUMULATION

Introduction

Much of the evidence of Se toxicity to aquatic biota is based either on observations of wild populations with unknown exposure history or on results of laboratory tests which may not adequately represent conditions in the field. In the field, variation in levels of Se amd other contaminants in water and food make it difficult to link Se residues and effects in fish populations with specific exposure conditions. Laboratory toxicity studies have enabled researchers to link effects to known exposure regimes, but laboratory test waters and diets may not include important components of natural Se exposures.

The objective of our studies is to relate Se concentrations and speciation in water and food to Se residues in aquatic organisms. Our approach has been to examine the formation and persistence of Se species in simulated complex ecosystems (microcosms) and to determine the bioconcentration of Se species from water and food in a laboratory food chain.

Methods

Closed-system microcosms (Huckins et al. 1984) containing water, sediment, and biota are being used to monitor Se distribution, bioaccumulation, and speciation under a variety of physicochemical and biological conditions. Radioactive ^{75}Se is measured in water and volatiles during microcosm studies and is quantitatively recovered from all microcosm components at the end of each study. Water samples from microcosms are further separated into several dissolved inorganic and organic fractions using chromatographic and solvent-extraction techniques.

The interactions of aqueous and food-borne uptake of Se are being studied in a laboratory food chain consisting of algae (Chlamydomonas reinhardtii), daphnids (Daphnia magna), and fish (bluegill, Lepomis macrochirus). Uptake of dissolved Se compounds (selenate, selenite, and seleno-L-methionine) at three dose levels is measured in algae during 48-hr static exposures and in daphnids during 96-hr static/renewal exposures. Daphnids in 14-day feeding exposures are fed batch algae cultures dosed with the same Se compounds and concentrations used in aqueous exposures. Bluegill exposures will compare Se uptake from dissolved selenite and Se-methionine in a static/renewal system; from these compounds processed through the algae-daphnid food chain; and from a combined food+water exposure.

Progress

Microcosm studies have focused on differences in the fate and bioavailability of dissolved Se species. The inorganic Se species selenate and selenite are more persistent in the water column than Se-methionine, but Se-methionine was preferentially accumulated by microcosm biota. A large percentage of added Se-methionine was also lost to volatilization, apparently due to biooaccumulation and production of volatile degradation products. Results of a speciation study with selenite-dosed algae suggest that release of volatile organoselenium compounds (probably methyl selenides) is associated with rapidly growing algal populations, whereas dissolved organic Se species accumulate during algal senescence and decomposition. This biologically-mediated cycling of inorganic and organic selenium species may contribute significantly to Se bioavailability in contaminated aquatic ecosystems.

Food-chain studies have examined the interactions of aqueous uptake and food-chain transfer of Se in algae and zooplankton. Se-methionine was more strongly concentrated from water by algae and daphnids than selenate or selenite (Fig. 1). Bioconcentration factors (BCFs) for Se-methionine in algae and daphnids were 2-100 times greater than corresponding BCFs for selenite or selenate. Selenite was more readily bioaccumulated by algae and daphnids than selenate from low dissolved concentrations, with selenite BCFs greater than 1000 at 10 μg/L or less, but uptake of these species was more similar at higher Se concentrations. Algae accumulated greater Se residues than daphnids at all doses of selenate and selenite tested.

In feeding studies, daphnids accumulated Se concentrations approximately equal to those in their algae food source over a wide range of algal Se concentrations (Fig. 2). Although daphnid uptake of Se from food was slower than uptake from water, food-borne transfer of Se may produce equal or greater Se concentrations in daphnids than uptake from water, due to greater bioconcentration of aqueous Se by algae. Daphnid Se uptake was not affected by the dissolved Se species used to dose the algae food source, suggesting that algae transform accumulated Se into equally available form(s) regardless of its chemical form in water.

Work remaining

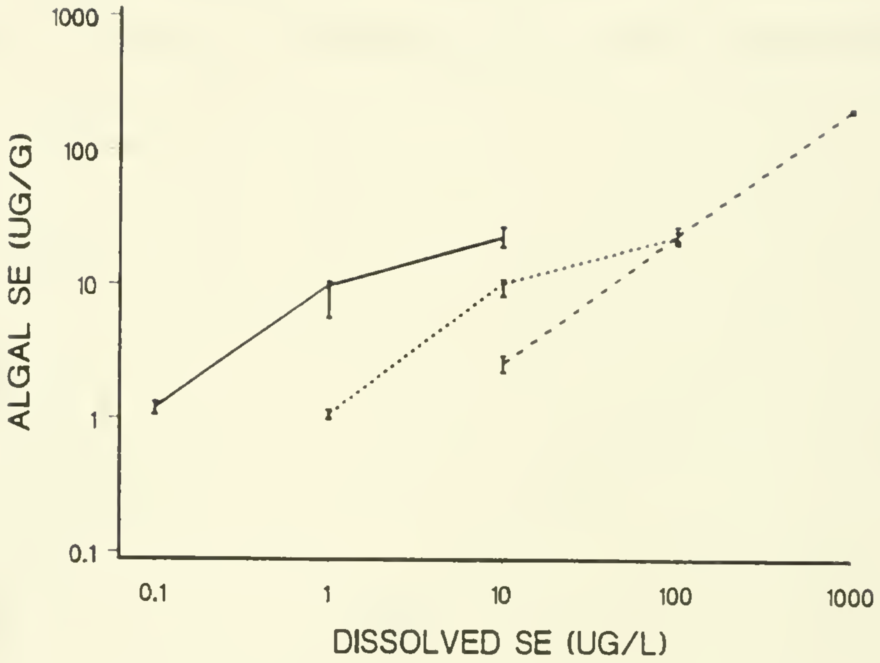
Remaining microcosm studies will further investigate the formation of organoselenium compounds, especially amino-Se moieties, in an attempt to validate the use of selenomethionine as a model organoselenium species toxicity and bioaccumulation tests. The first study will monitor production and release of organic selenium from selenite-treated algae during growth, daphnid grazing, and microbial decomposition. A second study will assess the effects of sediments and associated microfauna on sorption and cycling of dissolved organic selenium.

Food-chain studies will be completed with a series of aqueous and food-borne Se exposures with bluegill (*Lepomis macrochirus*). These studies will determine the effects of Se speciation, dose level, and exposure route on bluegill Se bioaccumulation. Results of these studies may be used to validate results of toxicity tests using artificial Se-enriched diets or to estimate food- and water-borne Se exposures required to produce toxic Se residues in natural fish populations.

Reference

Huckins, J.N., J.D. Petty, and M.A. Heitkamp. 1984. Modular containers for microcosm and process model studies on the fate and effects of aquatic contaminants. Chemosphere 13: 1329-1341.

Figure 1. Bioaccumulation of aqueous Se species by algae (*Chlamydomonas reinhardtii*) and daphnids (*Daphnia magna*). Se concentration in organisms (mean ± SE) plotted against nominal dissolved Se concentration. Lines connecting means indicate Se species: solid=Se-methionine; dotted=selenite; dashed=selenate.


ALGAL SE (UG/G)
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100
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0.1
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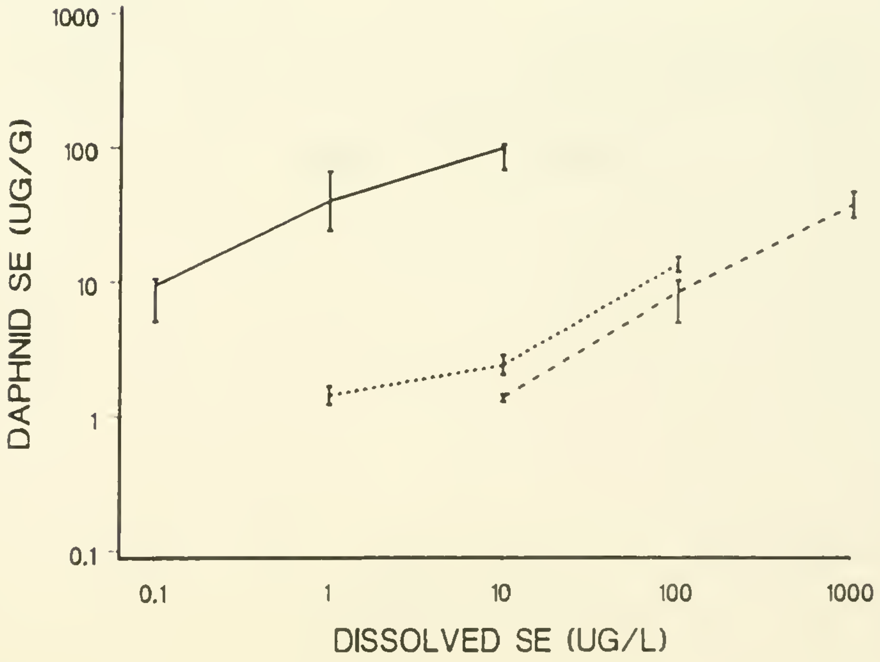
100
1000
DISSOLVED SE (UG/L)


DAPHNID SE (UG/G)
1000
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1
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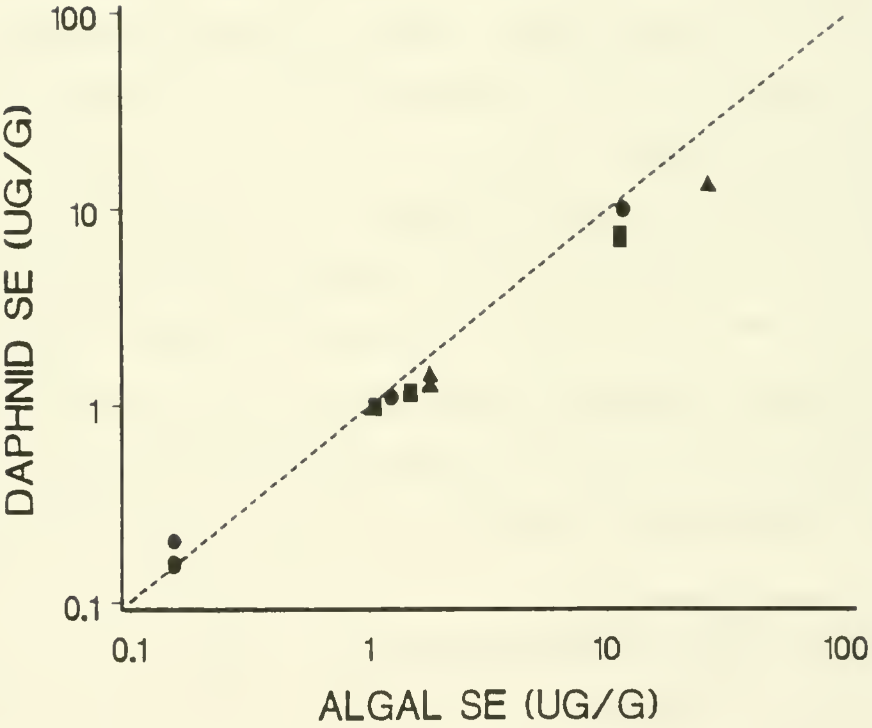
100
1000
DISSOLVED SE (UG/L)

Figure 2. Bioaccumulation of Se by *Daphnia magna* from algae (*Chlamydomonas reinhardtii*). Mean Se concentration in daphnids plotted against mean concentration in algal food. Dashed line indicates concentration factor (daphnid Se/algal Se) of 1. Symbols indicate Se species used to dose algae: circles=Se=methionine; squares=selenite; triangles=selenate.


100
10
1
0.1
DAPHNID SE (UG/G)
0.1
1
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ALGAL SE (UG/G)

BIOACCUMULATION OF CONTAMINANTS IN AQUATIC FOOD CHAINS AND FISH FROM THE SAN JOAQUIN VALLEY

Introduction

Several trace elements (e.g., boron, molybdenum, and selenium) occur at high concentrations in agricultural subsurface (tile) drainwater from the San Joaquin Valley. All of these elements are toxic to aquatic organisms when present in tile drainwater at concentrations above which they (the organisms) can safely metabolize and excrete. Some of these elements (e.g., selenium) are also known to bioaccumulate in organisms through the aquatic food chain.

The purpose of this field survey is to determine if trace elements are bioaccumulating to potentially toxic levels in aquatic food chains that receive markedly different exposures to tile drainwater. The field data will also be used as benchmarks for laboratory dosing studies conducted by NFCR-Columbia that examine the toxicological effects in fish fed contaminated foods.

Methods

Samples of water, sediment, detritus, algae, net plankton, aquatic macroinvertebrates (chironomids, amphipods, and crayfish), and fish (mosquitofish, bluegills, and largemouth bass) were collected in the spring (February-May) and fall (September-November) of 1987. The spring and fall collections represented high and low flow conditions, respectively, in the San Joaquin River. All collections were made from three streams that do not receive tile drainwater (i.e., east-side tributaries that drain the Sierra Nevada), two streams that receive variable amounts of tile drainwater (i.e., west-side tributaries that drain the Grassland Water District), and three sites on the San Joaquin River. The San Joaquin River sites were situated as follows: the first site was located upstream from the confluences with the tile drainwater-contaminated west-side tributaries; the second site was located downstream from the west-side tributaries, but upstream from the tile drainwater-free east-side tributaries; and the third site was located downstream from both the west-side and east-side tributaries. All samples were intended to be analyzed for six trace elements (arsenic, boron, chromium, mercury, molybdenum, and selenium). Additional samples of bluegills and largemouth bass were collected to document their food habits.

Progress

A total of 342 samples were collected in the spring, whereas 346 samples were collected in the fall. All samples have been shipped to NFCR-Columbia, where most are being processed and analyzed for trace elements. Due to a recent budget reduction, however, all samples from one of the east-side tributaries are no longer scheduled for analysis. In addition, the remaining samples will be analyzed for only three of the most important trace elements (i.e., boron, molybdenum, and selenium). The documentation of fish food habits is about half completed.

Work Remaining

After the chemical residue data are received, they will be examined in order to identify any geographic, seasonal, and "food chain" patterns in the concentrations of trace elements. These data will also be compared to findings from on-going "cause-and-effect" laboratory studies of fish fed contaminated diets in order to determine if the elemental concentrations in food chains from the San Joaquin River are potentially toxic. The documentation of fish food habits will be used to verify that the aquatic organisms collected for analysis of trace elements are indeed important components of the food chain in the river.

CHEMICAL SPECIATION OF SELENIUM

Introduction

The National Fisheries Contaminant Research Center is currently conducting research on the bioaccumulation of various forms of selenium in fish exposed to water of different synthetic matrices. There may be as many as three specific different forms of selenium in the exposure water: selenate, selenite, and selenomethionine. Conventional methods of preparation and analysis of selenium will not allow for the determination of these three distinct forms from a water sample. The methods designed for speciation of two of the compounds, i.e., selenate and selenite, will effectively destroy some to all of the selenomethionine present. Thus, speciation methods must be designed which will selectively separate selenomethionine from selenate/selenite species.

There is currently no information in the literature as to the storage characteristics of selenomethionine either by itself or in combination with selenate/selenite species. Thus, a long term storage study which investigates the variables of container material, water matrix, and temperature is necessary to validate the chosen method of preservation and storage for water samples collected from diluter exposure experiments.

Methods

To support U.S. Fish and Wildlife research designed to better define the ecosystem cycling of selenium in the contaminated Kesterson National Wildlife Reservoir, NFCRC chemists will develop analytical procedures to isolate and measure (speciate) various forms of dissolved selenium in water. Analyses of dissolved selenium residues in diluter water and natural river/reservoir water will require separate selenium speciation techniques. The diluter exposure water is prepared from well water subjected to reverse osmosis, followed by passage through activated carbon and mixed (anionic and cationic) resin beds, then formulated to provide a simulate San Joaquin River water. This water is subsequently fortified with selenate (Se^{+6}), selenite (Se^{+4}), various ratios of selenate and selenite, and selenomethionine. Natural waters may contain any of these forms of selenium as well as other unknown forms.

Using wet chemical techniques (1, 2), selenium can be speciated into three classes or forms: selenate, selenite, and the organoselenium class, which includes both Se^{o} and Se^{-2}. The chemical techniques employed are designed to fit the determinative approach, which is most often hydride generation atomic absorption spectroscopy. This type of detection is only sensitive to selenite. When one analyzes a water sample "as is" (no prior preparation), the results will be indicative of the amount of selenite in solution. Typically, a second aliquant of water is then taken and subjected to a hot HCl reduction procedure, which reduces any selenate in the sample to selenite, giving the selenite + selenate results. By performing a total selenium analysis (boiling with potassium persulfate to oxidize all selenium species to selenate, then hot HCl reduction to convert selenate to selenite) on yet a third aliquot of the water sample, the organoselenium class can be determined by difference.

Since the hot HCl reduction procedure will destroy selenomethionine, (convert selenomethionine to measurable selenite), water samples that contain this compound must be speciated in a different manner.

Free amino acids have classically been separated and characterized by passing the solution containing these free amines through a Cu-Chelex ligand exchange resin column (3, 4, 5, 6). However, no one has ever characterized this exchange resin for selenomethionine. We propose to produce the Cu-Chelex resin with 200 - 400 mesh material, which provides a superior ligand exchange and selenium capacity over the larger mesh sizes. Once produced, columns containing 5-mL bed volumes of CU-Chelex will be made and characterized as to optimum flow rate and chromatographic break point of selenomethionine. Experiments will be conducted to assure that Se^{+6} and Se^{+4} do not interact with the Cu-Chelex material in any way.

With quantitative selectivity of Cu-Chelex for selenomethionine, the overall speciation approach will be as follows: Diluter exposure water - an aliquot of the water will be passed through the Cu-Chelex column and the eluant collected. It is expected that (1) the Cu-Chelex resin will quantitatively retain all selenomethionine, and (2) the inorganic selenium forms (selenate and selenite) will be quantitatively recovered in the collected eluant. The eluant will be split, with half being analyzed by hydride generation atomic absorption to determine the Se^{+4} concentration. The other half of the eluant will undergo hot HCl reduction to provide the Se^{+4} and Se^{+6} results. The selenate concentration (Se^{+6}) can then be determined by difference. Another aliquot of the water is then digested to determine the total selenium concentration.

From these above concentrations, the selenomethionine concentration can determined by difference. This, of course, assumes that no additional unmeasured forms (species not transformed to selenite by these procedures) of selenium are present in the eluant. In practice, replicate water samples from the earliest and latest sampling times (using water from the highest exposure tanks) will be treated with Cu-Chelex resin and the eluant divided into thirds. The first two portions (each 1/3 of the eluant) will be either analyzed directly or treated with hot HCl reduction to determine the amounts of selenite and selenate in the eluant. The third portion of the eluant will be analyzed for total selenium, and the difference will be indicative of other unknown forms of selenium that may not be trapped by the Cu-Chelex column, or species not transformed to selenite by these procedures. Finally, a separate portion of the untreated water sample (not subjected to Cu-Chelex separation) will be digested to determine the total selenium concentration in the untreated water. From the above concentrations, the selenomethionine concentration will be determined by difference.

Although there are considerable literature references for the preservation of selenate and selenite, there is little information regarding preservation of organoselenium compounds, either by themselves or in combination with selenate and selenite. Thus, we propose to determine the preservation behavior of three forms of selenium (selenate, selenite, and selenomethionine) under two sets of preservation conditions. The following table depicts the preservation experimental design:

Conc. of $Se^{+6,+4,-2}$ (ppb)	Bottle Material Polyethylene (-20°C)	Glass (+4°C)
10	xxx	xxx
50	xxx	xxx
100	xxx	xxx

Eighteen bottles, nine constructed of conventional polyethylene and nine of Wheaton "400" brand borosilicate glass (all 500 mL size) will be used in the experiment. All bottles will be filled with 500 mL of equal concentrations of selenate, selenite, and selenomethionine, at three different concentration levels: 10, 50, and 100 ppb. All solutions will be acidified by making up in 1% HCl. The polyethylene bottles will be stored at -20°C and the glass bottles at +4°C. All solutions will be analyzed at Day 0, 7, 30, 60, and 120. Thus, for each analysis event, there will be 9 bottles X 2 types X 3 aliquots of solution, for a total of 72 analyses.

For all 5 analysis event days, there is a total of 270 analyses prior to Day 0, the selenomethionine standard will be analyzed with an amino acid analyzer to assure that this compound has not experienced breakdown.

Progress

All experimental work has been completed. The results found can be summarized as follows:

-- In an acidified (1% HCl) high ionic strength matrix, selenate, selenite, and selenomethionine can be sucessfully preserved and stored for at least 4 months without any significant changes in concentration taking place.

-- In an acidified matrix alone (only 1% HCl), selenate and selenite underwent changes after 60 days of frozen storage in polyethelene containers. The selenite concentrations decreased, and selenate increased, while selenomethionine concentrations remained about the same. The same experiment, but using refrigerated glass containers, produced no significant changes over the same time period.

-- Selenate, selenite, and selenomethionine can be sucessfully separated and quantified by the use of Cu-Chelex ligand exchange chromatography coupled with hydride generation atomic absorption spectrophotometry.

Work Remaining

Two manuscripts are in preparation which summarize the findings of this study.

Wiedmeyer, R.H., and T.W. May. Speciation of selenium forms with copper chelex ligand exchange chromatography.

Wiedmeyer, R.H., and T.W. May. Effects of acidity, container material, temperature, and ionic matrix on storage of selenate, selenite, and selenomethionine.

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